



11 May 1978

Another year of armaments

THE annual appearance of *World Armaments and Disarmament* from SIPRI, the Stockholm International Peace Research Institute, has in recent years drawn a mixture of admiration that so much is done by such a small operation, concern at the relentless pace of armaments and the almost imperceptible motion of disarmament and, at least from us, nagging worries that there may be pressures on SIPRI, from within or without, to take a more active role in speaking up against armaments and for disarmament. This worry is not because we disagree with such objectives—it is simply that in a field where information is scarce and intelligent extrapolations necessary, preconceptions can drastically alter conclusions. This year's volume (published by Taylor & Francis, London, at £18.00) carries on in the same valuable tradition, again puts those who are interested in military matters in SIPRI's debt, and again raises those nagging worries.

Last year the total world military expenditure was getting on for \$400 billion. In real terms this figure has risen 80% in the past 20 years. But this rise has been by no means uniform—since 1967 the total has grown by only 13%, and at present climbs by around 1% per year. NATO and the Warsaw Treaty Organisation in fact contribute almost no growth; it largely comes from the vigour with which the Third World has acquired arms. In twenty years the Middle East has multiplied its arms expenditure twentyfold, though conceivably this grotesque expansion is now at an end. Maybe China is the next expansion area—for all the political storm concerning remarks that Britain's Chief of Defence Staff, Sir Neil Cameron, made in Peking about Britain and China's common "enemy at our door", it was his comments in Hong Kong that should have been widely quoted. For he spoke quite openly about China's need to move from low to high technology, and left no doubt that the armaments manufacturers of the West should be happy to take part in such a profitable operation.

The strategic arms limitation talks, SALT 2, first mooted in December 1974, have still not produced any final agreement, even though SALT 1 has expired. The problems seem to be mainly in how to treat cruise missiles and the Russian supersonic bomber, although recent reports suggest that enough progress has been

made for a treaty to be signed this summer. But, as SIPRI points out, any treaty is unlikely to affect either side's arsenal in a quantitative way; the numbers of missiles, bombers and submarines permitted are likely to be pretty well what both sides want. This focuses attention on whether SALT 2 can have any qualitative impact, maybe by ruling out certain new types of missile. One of the disappointments of SALT has been that the talks have ended up seeming to authorise new qualitative developments that each side wanted and proposing numerical limits with which they were happy to live. Could a new round bring anything different?

SIPRI would like to see a clampdown on mobile inter-continental ballistic missiles. These have no fixed abode and so are extremely difficult to eliminate. A US version under study at present, the M-X, could come in one of two varieties. Either a missile base could contain many silos which were regularly loaded and unloaded with missiles or decoys, making it impossible to tell from satellite reconnaissance which particular silos are worth attacking. Or an underground network of tunnels perhaps thirty kilometres long and fitted with blast doors would contain missiles on trucks. If the need arose, the missile would be pushed up through the soil and fired. Attack of the base would be almost pointless as reconnaissance could not reveal the location of trucks at any particular time.

Is this new generation of mobile missiles a bad thing? Clearly it is in all sorts of ways—vertical proliferation, yet more expenditure and so on. But should it be legislated out of existence by SALT? SIPRI says that it should, as it would "not only destabilise . . . the strategic balance . . . but also seriously complicate the negotiation of future strategic arms limitation agreements". In this SIPRI could be wrong. Up to the present submarines have fulfilled a similar role of an almost invulnerable force, but improvement in anti-submarine warfare techniques are slowly eroding this. There is a possibility that late in the century nations will no longer be able to count on their submarine forces for some form of ultimate reassurance. Maybe if both superpowers agreed to develop mobile ICBMs in a controlled and measured way, such a force would take over this role and conceivably stabilise rather than destabilise. □



Glacier 'retreat' threatens Alaskan oil tanker route

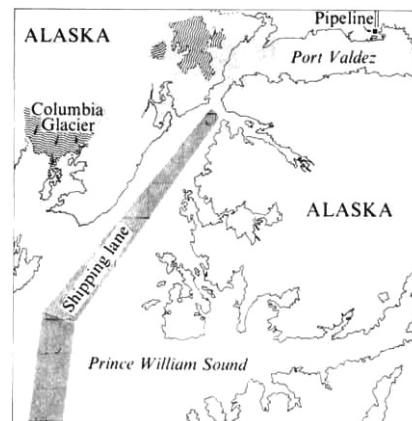
A MAJOR glacier is threatening to disrupt the transportation of oil from Alaskan oil-fields by discharging large masses of ice into the shipping route used by supertankers, according to scientists of the US Geological Survey. The geologists have mounted a research effort into the state and behaviour of the 425-square-mile Columbia glacier, which discharges into Prince William Sound 40 kilometres from the end of the Trans Alaskan pipeline at Port Valdez.

They are concerned that the glacier could, as a result of changing climatic conditions, experience a 'drastic retreat' which might result in the discharge of about 50 cubic miles of ice over a period of 30–50 years—and consequently a major hazard to shipping. Coastguard officials admit that the dangers are "potentially very serious". They are already studying various contingency plans, in particular the use of a three-mile nylon

cable to keep the icebergs out of shipping lanes.

Glaciologists have known for many years that glaciers, which normally deposit ice into the sea at a relatively slow rate through a process known as 'calving', can suddenly become unstable and discharge large amounts of ice over a relatively short period of time.

Only in recent years, however, since the route of the 800-mile Alaskan pipeline was planned and agreed upon, have the climatic mechanisms leading glaciers to behave in this way begun to be understood. In particular, drastic retreats are thought to follow a period in which the glacier becomes thinner due to a temperature imbalance between the ice and the surrounding atmosphere. And this thinning is precisely what has been observed on the Columbia glacier, so far the only glacier along the Alaskan coastline which has not experienced a



The threat to shipping lanes

drastic retreat in the past one thousand years.

Since 1974, when the possible implications were first pointed out by glaciologist Dr Austin Post, government geologists have been looking at the behaviour of the glacier. A current project to collect data during the year September 1977 to September 1978 at a cost of \$800,000 has involved considerable reallocation of

A bad year for weather, a good one for climate

David Dickson discusses the initiatives contributing to a US programme for climate research

TWO SEVERE winters and a period of prolonged drought in California have, like the proverbial hangman's noose, had a wonderfully concentrating effect on the collective minds of US political administrators.

The net result has been a major boost, both politically and financially, for climate research. And accompanying this has been a perceptible shift in the balance of research policy away from attempts to achieve increasingly accurate forecasting towards more general concerns over the nature and effects of climatic change.

The two houses of Congress have already passed broadly similar bills, the first going through the House of Representatives last autumn and the second passing through the Senate two weeks ago. With few differences separating the two sides, early agreement seems a distinct possibility, and a National Climate Program Act should be ready for the President's signature within a few weeks.

The administration has itself been developing a parallel initiative. In his Budget request for 1979, presented to Congress in January, President Carter suggested the outlines for a broad inter-agency climate programme, based largely on the results of a study carried out last summer by an inter-agency

panel reporting through Dr Frank Press, his science adviser.

Under these proposals, the total amount of money spent by the various agencies on climate issues would be increased from \$75.7 million in 1978 to \$104 million in 1979. And the Department of Commerce's National Oceanic and Atmospheric Administration (NOAA), would be the "lead agency" for such a programme.

Nor has the scientific community been slow to respond. The National Academy of Sciences has established a Climate Research Board under the chairmanship of Dr Robert White, a former director of NOAA. This will act as a focus for the climate interests of a wide range of NAS committees, and is already engaged in a study of the research efforts of the various government agencies.

The various developments now fitting together into a single picture are the result of a concern for climate which has been growing since the beginning of the decade, and of various political initiatives which originated in the Nixon era, but became bogged down because of Watergate.

In particular, they reflect a shared shift in philosophy—and hence in research methodology—away from a prime emphasis on meteorology and on

achieving increasingly sophisticated and accurate weather forecasting, a field which many feel has failed to live up to early expectations.

(This failure has itself given rise to new strategies. Thus forecasters, for example, are increasingly expressing predictions in statistical terms, such as announcing that "the chance of precipitation tonight will be 100%". And agriculturalists are shifting attention to the diagnosis of existing weather patterns and their likely impact on crop yields).

A second reason for the increased emphasis in climatic change has been a growing awareness of the effects of the interaction between social activities and the atmosphere. Climatologists such as Stephen Schneider at the National Center for Atmospheric Research, for example, have argued that, as the world moves close to the limits of its capacity for producing food, water and energy, so the apparently marginal effects of climate behaviour—such as the Sahelian drought or crop failures in India and the USSR—become correspondingly significant.

Concern at the long-term implications has therefore focused on two closely-related areas, in both of which the complexity of the problems is



Columbia glacier: slipping into the sea

research funds within the US Geological Survey.

In a report which has just been published, the Department of the Interior says that last summer a considerable increase in the number of small icebergs deposited by the glacier into the shipping channel caused night-time shipping to be suspended during part of August. Commenting on the report, Dr Mark F. Meier, chief

of the USGS glaciology project, said: "There is no evidence yet that the Columbia glacier has become unstable and begun a drastic retreat, but last summer's experience indicates that it needs to be watched closely."

According to Dr Meier, since 1975 iceberg discharge has increased from one summer to the next, while the altitude of the ice surface near the discharge point decreased by more than 30 feet between 1976 and 1977. "This may mean that a drastic retreat is imminent," he says.

The geologists claim that one reason the glacier has not retreated so far is that its front end rests on a raised edge, behind which the glacier is thought to be as much as 2,000 feet deep for up to 20 miles.

"If huge icebergs do form, the water over the moraine where the glacier rests is so shallow that the larger icebergs will be unable to leave the embayment. These large bergs would break up into smaller bergs which could then escape," according to Dr Meier. Such icebergs could, given winds from the appropriate direction, float into the shipping

lanes; the scientists claim that this would raise the hazards to shipping by a factor of 10 or even 100 times.

The US coastguard has been discussing various contingency plans, the most feasible of which appears to be a proposal to restrain the icebergs using a bundle of 13 nylon ropes, each 10 inches in diameter. Such a project would, it is estimated, cost \$30 million; but it is pointed out that this is less than the market value of three days' supply of oil from the end of the pipeline.

So far the oil companies have, in public at least, adopted a 'wait and see' attitude. A spokesman for Alyeska, the firm which operates the pipeline, said last week that the glaciologists' predictions remained theoretical and speculative, and that the company saw "no evidence" that a major retreat was imminent. However, the spokesman admitted that if such an event did occur, then it might present a major problem to the orderly transport of oil from the Alaskan fields.

David Dickson

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Camera Press

The effects of extreme weather: flood and drought

reflected in the amount of research needed to tackle them: the impact of human activity on climate change, and conversely the impact of such change on human activity.

"The effects of social activity on the climate is an area which we still know very little about," says Dr Francis Bretherton, director of NCAR, a research centre run jointly by 40 universities primarily on National Science Foundation funds.

"In some areas, physical scientists are making a lot of assumptions which are almost certainly wrong. There are many important questions concerning the trace chemistry of the oceans and the atmosphere, for example about the carbon, nitrogen and sulphur cycle on which much basic research remains to be done."

Similar needs are also being recognised in climate dynamics, until

recently a relatively neglected field. And in a recent report on research strategy for the US climate programme, the US Committee for the Global Atmospheric Research Programme (GARP) suggested that high priority be given to detailed studies of the annual cycle of the atmosphere and its lower boundaries.

The future of funding in universities for climate research looks promising. Admittedly the increase in the National Science Foundation's budget for climate research requested by the President for 1979 is not as large as for other agencies; however these have been told that a significant proportion of their research funds must be spent in universities.

There remains one area of uncertainty: the scientific and political strategies that should be adopted for assessing the impact of climate change

on social activity—and thus on policy formulation.

Related directly to this is the problem of co-ordinating the research programmes of a number of different agencies. While pursuing different mandates, each is required to tackle issues that involve a complex measure of science and technology with fields such as geography, ecology, agronomy, economics, political science and even anthropology.

The congressional point of view is that the philosophy of a national climate programme should essentially be "user-pull" rather than "researcher-push". In line with this, both of the current bills suggest that prime responsibility should rest with the head of the lead agency (in the Senate Bill the head is explicitly stated to be the Secretary of Commerce, responsible for NOAA).

Nuclear power uneconomic says congressional committee

THE House of Representative's committee on government affairs has published a damning indictment of claims that nuclear power is a cheap form of energy, claiming this only to be true if hidden costs such as federal subsidies and the costs of waste management are ignored.

The report, which was made public last week and is based on hearings held last autumn by the committee's subcommittee on the environment, energy and natural resources, states unequivocally: "Contrary to widespread belief, nuclear power is no longer a cheap energy source".

It continues: "In fact, when the still unknown costs of radioactive waste and spent nuclear fuel management, decommissioning and perpetual care are finally included in the rate base, nuclear power may prove to be much more expensive than conventional energy alternatives such as coal".

And the report adds that nuclear power may not either be economically competitive with safe, renewable resource energy alternatives such as solar power.

The report has already raised fierce controversy in Congress. Many members of the committee have recorded dissenting views, some claiming that although it purports to be concerned with the more efficient management of the nuclear power programme, its whole tenor is anti-nuclear. Others claim that it takes a superficial look at many complex scientific and technical issues.

However in the present political climate, the report provides a powerful political weapon in the hands of those who argue that the apparent economic unattractiveness of alternatives such as solar energy is a result of distortions due to federal subsidies of the nuclear programme.

Stating that both Congress and the

American people should be aware of the full implications of the nuclear option, the report points out that although there was initially a very rapid growth in the building of reactors up to the early 1970s, a variety of economic, technological and safety issues had since contributed to a sharp fall-off in orders.

Among the factors leading to increased estimates of the costs of nuclear power, the report lists the facts that:

- construction costs for a nuclear plant are increasing more rapidly than the general rate of inflation, or 10 times faster than those for an oil refinery

- nuclear plants are experiencing serious cost over-runs—as much as 267% for one plant

- the cost of uranium has risen from \$7 per pound in 1973 to more than \$40 per pound today for new sales

- various "hidden costs" and "extra costs", such as federal subsidies for nuclear power research and development, and limited liability and insurance are not incorporated in electric rates

- unknown costs, such as those associated with the "back end" of the fuel cycle, have not been determined, and are not incorporated in the price of electricity.

The report concentrates in particular on the difficulties of predicting the costs of storing radioactive waste, pointing out that Department of Energy estimates for disposing only military wastes had ranged from \$2 billion to \$20 billion. Similar uncertainty exists about the costs of decommissioning power plants.

The report recommends a number of actions which the Department of

Energy should undertake to gain the necessary technical and economic information about waste storage. And it concludes by suggesting that Congress should consider repealing the Price-Anderson Act and allow the nuclear industry to assume full liability and responsibility for the safe operation of commercial power plants.

Meanwhile support for the technical potentiality of moving into a non-nuclear future has come from another source, a study carried out jointly by the University of California and two of the Department of Energy's national laboratories, Lawrence Berkeley and Lawrence Livermore Laboratories.

Their preliminary report on the potential of energy systems using decentralised technologies to meet California's future energy needs was published at the end of last month. The report was prepared as part of the Department of Energy's response to the challenge laid down eighteen months ago by Amory Lovins on the possibility of moving from "hard" to "soft" energy paths.

Although the report does not look at the economic aspects of changing to different energy technologies, it concludes that soft technologies such as sun and hydro-power, wind, biomass and geothermal energy might be able to meet most of the state's needs by the year 2025.

Making various assumptions about the future development of Californian society—such as an approximate doubling of population, tripling of total economic activity and quadrupling of energy prices in 2025—the report says that "analysis indicates that it is possible in purely technical terms, to come quite close to operating the postulated advanced, post-industrial society in California using indigenous, sustainable resources".

David Dickson

Sakharov speaks in support of nuclear power

"DEVELOPMENT of nuclear energy is an indispensable condition for the maintenance of economic and political independence," according to Academician Andrei D. Sakharov.

Writing in the *Neue Zürcher Zeitung*, Sakharov expresses his alarm at the "numerous noisy demonstrations" in the West against the building of nuclear power stations. For western Europe and Japan, with their dwindling supplies of fossil fuels, the alternative to nuclear power is "dangerous" economic dependence on "energy supplies in the form of fossil fuels from the USSR or from countries under its

influence which produce oil and natural gas".

By threatening to withhold energy supplies, he says, the Soviet Union could win for itself political concessions, each leading "inevitably" to the next, with a final outcome that is "very difficult to predict".

Explaining Soviet policy, Sakharov recalls a conversation which he had in 1955 with "a high Soviet official" who implied that Soviet policy in the Middle East was supporting Nasser so as to cause an oil shortage in western Europe. Today, he says, "the situation is very complicated and has many

facets", but the parallel remains. The Soviet Union still has an interest in "profiting from the energy weakness of the West".

Regarding possible Soviet inspiration behind the campaigns, Sakharov is non-committal. "We know of no facts about this," he says. Nevertheless, in his opinion, "the widespread prejudice against nuclear energy and the lack of insight concerning the inevitability of the atomic age" mean that, if there should be some Soviet influence involved, only a very slight effort would be needed.

Vera Rich

US halts search for extra-terrestrial intelligence

A US congressional subcommittee has severely cut back a request from the National Aeronautics and Space Administration for funds to begin a search for extra-terrestrial intelligence, thus according to NASA officials effectively 'killing' the project.

The subcommittee has also denied the agency's request for funds that would allow the Space Shuttle to prevent the descent and disintegration of Skylab, on the grounds that current delays in the Shuttle development programme put the success of such a mission in doubt. And as a further consequence of the shuttle delays, the subcommittee has directed NASA to set up a 'contingency fund' of \$30 million to cover any extra costs that the shuttle programme may incur. This money would be taken from the funds for three major space science projects which have already been requested for the fiscal year 1979: the Solar-Polar Orbiter Mission (cut back by \$5 million), the Large Space Telescope (cut by \$15 million), and the Jupiter Orbiter Probe.

Scientists regard the most critical of these as being the possible cut in the budget for the Solar-Polar Orbiter Mission. A one-year delay in funding

could mean a three year delay in the project until the Earth, and Jupiter (whose gravitational pull is necessary to send the spacecraft over the poles of the Sun) are once again in a favourable position relative to one another.

The effect of these cuts, if they are left standing when the NASA budget request is discussed by the full House, could be to set back significantly each project (although delays in the Shuttle programme would inevitably

result in delays in launching the Space Telescope, which is to be one of the Shuttle payloads).

The budgetary decision was made by a subcommittee of the House Committee on Appropriations under the chairmanship of representative Edward P. Boland. Last year the same subcommittee recommended that funds be withheld for a start to the Jupiter Orbiter Probe in 1978, a decision only overturned on the floor of the House after an intense lobbying effort by the space science community.

The subcommittee has recommended that the budget for the search for extra-terrestrial intelligence (SETI project) be reduced to \$600,000 from a requested \$2 million. "This is not enough even to start design work on the antennae," a NASA official said last week.

As regards the cut in NASA's request for funds to boost Skylab back into an orbital path, the subcommittee has said that it would be prepared to consider an additional request for funds to do this if current attempts to reactivate Skylab's engines, and thus keep it in orbit until Space Shuttle is ready, are successful.

David Dickson



"Close down encounters of the third kind"

Copernicus Astronomical Center opens in Warsaw

THE OFFICIAL opening, on 24 May 1978, of the Copernicus Astronomical Center in Warsaw marks the beginning of a new era of collaboration between theoretical astronomers from eastern and western countries. The institute promises to be an important meeting place, especially as Soviet astronomers are likely to find it easier to get to Warsaw than say Cambridge or Princeton. Furthermore it adds recognition to the outstanding advances made by Polish astronomers in the understanding of binary stars.

In 1970 Józef Smak, the present director, and Bohdan Paczynski set out proposals for an international centre that could be constructed at relatively low cost. They envisaged that such an institute could help Polish researchers keep in touch with one another, as well as providing good facilities for visitors from other countries.

At the outset there seemed to be no chance of getting the necessary finance. However, when Paczynski aired his ideas in the USA, colleagues suggested an approach to the National Science Foundation (NSF). This led the Polish Academy of Sciences to make a formal

proposal to the NSF. The aims of the project were evidently well received. Academy of Sciences to make a formal In 1973, the five hundredth anniversary of the birth of Copernicus, the NSF presented the equivalent of £1.4 million in Polish currency to enable a new institute to be founded for Polish astronomers.

The center includes a hotel capable of accommodating a dozen visitors and their families. This hotel will be valuable since the language and cultural difficulties that most scientists would otherwise experience are largely removed. An adequate budget will provide for the living expenses of western visitors; this is essential as the official exchange rate is unfavourable for long-term visitors.

Currently the office facilities are spacious but they will become rather cramped if a proposal to base a space research group there is carried out. A PDP 11/4S computer, purchased with a donation of \$100,000 is available for modelling stars and galaxies.

For many years the Warsaw school of astronomers has been at the forefront of thinking on the properties of

close double stars. Another important research area is that of stellar evolution, especially the study of instabilities, variations, and the effect of (hypothetical) highly-condensed cores on the evolutionary behaviour. A new field under development is that of high-energy astrophysics, particularly in the relativistic regime.

To improve contact between the fragmented schools of astronomy in Poland, the Copernicus Center will have a vigorous program of workshops, think-tanks, and graduate study program. It will also act as the main center for stellar evolution studies in eastern Europe.

What will be of special significance to visitors from the UK and USA is the possibility of collaborating with Soviet astronomers who normally find it very difficult to get permission to visit the west for extended periods. Polish astronomers have already made excellent contacts with researchers in other countries. Now they are in a good position to return hospitality and make further important contributions on an international basis.

Simon Mitton

Israel's scientist-President resigns

EPHRAIM Katzir, the distinguished biochemist, has resigned as President of Israel to be replaced by a career politician—Izchak Navon. Katzir was Israel's fourth president, and the second scientist to hold the office. (Dr Chaim Weizmann was the first, and also the first president.)

Katzir took the opportunity of a recent visit by foreign scientists to Israel to indicate areas in which Israel should increase her scientific effort: principally, pollution control and industrial research.

Ex-president Katzir is concerned about the pollution of the Mediterranean and the Sea of Galilee, and the River Jordan. Israel shares her interest in the Mediterranean with many other countries, but Galilee and the Jordan are—relatively speaking—local problems. Galilee, important for irrigation and fishing, is concentrating massive amounts of nitrate fertiliser, which runs off from the densely-packed agricultural holdings surrounding the lake. The Jordan is virtually dry due to the re-channelling of its waters, and is seriously polluted despite five or six years' effort to reduce the problem. The only pollution bright spot is industry, a fact Katzir attributed to Israel's lack of raw materials.

Katzir is returning to biochemical research—his lab remained empty while he was president—and hopes to help develop biotechnology and bio-engineering in Israel. In his view the country's applied science needs improvement.

Among others, Michael Feldman, Professor of Biology at the Weizmann Institute, agrees with Katzir. "Applied science does lag behind basic science" he told *Nature*. The anomaly of science in Israel is the great success of agricultural research, contrasted with the limited success of industrial research. Agriculture has always been an essential activity for the Jews, even predating the establishment of Israel as a state. Feldman points out that the early agronomists were able to enlist the help of scientists by clear presentation of well-defined problems—for example, those created by plant diseases. The symbiosis between agriculture and science has continued ever since.

Industry, however, has only developed during the past 20 years; it hardly existed during the early part of the century—and the embryonic Israeli industry was unable to define its problems.

Alastair Hay

THE great scientific debates continue. Recombinant DNA, ozone, nuclear energy, low-level insult, radioactive waste disposal, soft energy paths: they are constantly in the news.

Most of these issues lie at that ambiguous junction between science and society where science cannot give precise answers. Scientists with seemingly equal credentials often come to opposite scientific conclusions. Many of the questions are trans-scientific: they lie beyond the proficiency of science in principle. No wonder when debating these topics scientists sometimes represent as known what is only half-known, or apply lesser norms of scientific proof than they apply to conventional science.

Science, through its learned societies, has established the referee system to extirpate irresponsibility. It is hard to publish in the recognised journals of science, and rightly so. Even though a truly great idea is occasionally rejected, on the whole the system has maintained science as a responsible undertaking.

The system of peer review and refereeing is being undermined in many semi-scientific areas of inquiry. Take energy, a subject on which I spend most of my time. In the past few years no fewer than 40 journals in English devoted to energy and resources technology and policy have sprung up. I have no estimate of the number in other languages, but it is probably large. One need not read very far in this new literature to recognise that the refereeing is less strict than is the refereeing of the well-established disciplinary journals.

This is not necessarily the fault of the editor. It is rather that the fields are new, they involve many disciplines, and, as is so often the case with interdisciplinary investigations, the norms of acceptability are rather poorly defined. A physicist can hardly pass judgment on an economist, nor, for that matter, can the economist properly pass judgment on a physicist.

Can the scientific societies themselves, which have maintained the standards of responsibility within their own disciplines, help make these trans-scientific debates more responsible? Clearly, a good deal can be done. For example, the American Physical Society has now issued three major reports—on nuclear reactor safety, energy conservation, and nuclear fuel cycles and waste management. Each report as required intensive study by a dozen or so scientists, not all of whom are necessarily members of APS. Each report has been reviewed by a special Review

Trans-science



ALVIN M. WEINBERG

Committee of the American Physical Society, and in general, the scientific standards are high.

The best of the reports is the one on the nuclear fuel cycle and waste management. This is as careful and reasoned a scientific assessment of these touchy questions as exists. The group concluded that "For all LWR fuel cycle options, safe and reliable management of nuclear wastes and control of radioactive effluents can be accomplished with technologies that either exist or involve straightforward extension of existing capabilities".

These three reports, though generally, admirable, illustrate the difficulty when a society devoted to a single discipline tries to analyse an interdisciplinary issue. In both the reactor safety and the fuel cycle studies some assessment of the biological hazard of low-level radiation was essential. This lies outside the competence of physicists, yet neither the hazard of a reactor accident nor of the nuclear fuel cycle can be addressed without considering low-level radiation insult. The nuclear fuel cycle report to my mind exhibits a far more mature understanding of the matter than does the earlier report on reactor safety; yet I must concede that physicists talking about biology, however sensibly, are harder to believe than when they talk about radiation damage.

Despite this shortcoming, I believe the American Physical Society deserves great credit for undertaking serious studies of technologies that arise from physics. I view such involvement of established scientific societies in such trans-scientific questions as a hopeful way of injecting more responsibility into these debates. I hope other scientific societies will emulate the American Physical Society and help establish a more responsible technical literature in these controversial spheres of inquiry.

Vietnam calls its scientists to arms

VIETNAM hopes to have a "modern, scientific capability" in 15 to 20 years' time. This is the message which the government has relayed to the country's scientists. In return, the politicians expect the scientists to participate in a programme of reconstruction; science and technology are seen as the key to Vietnam's economic development.

Some scientific disciplines are better equipped than others to participate in the programme. In the past, the physical sciences received considerably more support than the biological sciences (see the first article in the series, 12 January, page 101). This will be remedied, however, in new allocations of funds. Support for agriculture and medicine is given high priority in Vietnam's new economic plan. It is the biologists who will be most actively involved in these two areas so they can expect more government finance in the future.

But the support could be some time in coming; Vietnam has virtually no extra resources to assist her scientists at present. Two disastrous harvests in the past two years have emptied the government coffers, and the government's dilemma is evident in the exhortations to the biologists to manage with the resources they already have.

Vo Nguyen Giap is the first Minister for Science and Technology, the one politician in Vietnam considered experienced enough to run the new ministry. Giap still retains his original portfolio, that of Minister of Defence, and it is apparent that he believes that some of the lessons learned in his years of military service are applicable to the problems of science. The biologists explained to him that they lacked resources in the form of equipment and chemicals and that this shortage would hamper their new research programmes. Giap replied with an analogy based on the Vietnamese army—a relatively unsophisticated body in terms of weaponry which still defeated a highly sophisticated American 'military machine'. "If the army could rise to the occasion then surely the scientists can do the same".

Some scientists, however, say that it is possible to do a certain amount of work using inexpensive equipment but for some programmes it is vital to have sophisticated technology. One research programme serves to illustrate their argument. Chemists and botanists are

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currently investigating plant sources used in traditional medicines. They hope to isolate the active components in the plants, identify them and if possible synthesise these ingredients chemically; the ultimate objective being to hand over the information to the pharmaceutical industry. To perform this schedule of work and provide the fine fingerprint of each active component it is necessary to have access to such techniques as nuclear magnetic resonance and mass spectrometry, neither of which is available in Vietnam. The final analyses are performed abroad at present and it is often two months before the results are available.

Building up biological research

But not all of the biologists have clearly defined research schedules. Many are now involved in detailed discussions on research required to assist agricultural development. Their views are as varied as their backgrounds, many having received their training in foreign countries. Most were trained in the Soviet Union, others in Eastern Europe, China, France or West Germany.

Dr Phan Thanh Luu, the only truly qualified biochemist at the Vietnam Scientific Research Centre in Hanoi, spent seven years in West Germany the last four of which were at the Max Planck Institute. On completion of his degree Luu was offered new research posts in Germany and the United States. He opted instead to return to Vietnam. Luu is one who believes passionately in the future of science in Vietnam. For him there was no dilemma involved in sacrificing a promising research career in the West and returning to help build biological research in Vietnam. He has no illusions about the difficulties faced by the biologists. He says: "We have to

General Giap, soldier turned scientist: "If the army could rise to the occasion, then surely the scientists can do the same".

Final part of Alastair Hay's series from Vietnam.

design a programme of research which will improve the efficiency of our agriculture. Unfortunately we don't all have the necessary experience, so it is no easy task". Luu maintains that in the immediate future better irrigation and the use of fertilisers could greatly improve crop yields. "So we are talking about research work that will only have an impact ten to fifteen years from now".

An obvious candidate for research is the floating fern *Azolla pinnata*. This is an important source of green manure and fodder for pigs, supplying about 20% of their protein requirements. In combination with a symbiotic blue-green alga (*Anabaena azollae*) it fixes nitrogen and for this reason is used as manure for rice. The crop grows readily in cool weather and about two-thirds is cropped weekly in winter. Maintaining a supply of fern over the hot summer as "seed" for the next season is still a problem. At one time the technique of doing this was jealously guarded from women in case they married outside the village and in passing on the knowledge ruined a valuable source of income from the sale of the "seed".

Luu acknowledges that research on *Azolla* is of considerable importance. Little is known about the biochemistry of ferns generally according to Professor N. W. Pirie, formerly of the Rothamsted Experimental Research Station in the UK, so a study of *Azolla* would be "an essentially Vietnamese matter". This is the kind of research which the government would finance readily.

Even if the biologists can justify their claim for more immediate funds and the government complies with their request there will still be a limit to the support the country can provide. The use of radioisotopes in research is something many biologists desire. But the technique requires a regular source of material and scintillation counters to perform the measurements.

This kind of equipment is simply out of the question at the moment. It might not have been so unrealistic had the United States not destroyed the one nuclear reactor in South Vietnam at the Atomic Energy Centre in Dalat 150 miles north-east of Saigon. When Dalat was in imminent danger of being cap-

Camera Press

tured by Communist forces in March 1975 the Pentagon acted swiftly to dismantle the reactor. US scientists were flown to Dalat to remove the reactor core and dynamite the buildings. The Pentagon justified its action on the grounds that the North Vietnamese would use the reactor for the manufacture of nuclear weapons. Yet the North Vietnamese insist that the Dalat reactor was designed for peaceful purposes. This is confirmed by a 1968 UNESCO publication on 'Science and Technology in Asian Development' which states that the reactor provided radioisotopes for use "in agriculture and medicine". Unless the function of the Atomic Energy Centre had seriously altered since 1969, many observers feel that there was little justification for the Pentagon action.

Discrimination against scientists

The Pentagon was not alone in voicing its fear about what would happen to South Vietnam when the Communists took Saigon. Many other governments expressed anxiety for the safety of residents in the city. But when it was realised that their lives were not in jeopardy a new fear rose like a phoenix to take its place: the fear of discrimination against those who supported the former president of South Vietnam—Thieu—either directly or tacitly.

The issue of discrimination against intellectuals and scientists was one I raised with Dr Tran Tri of the State Committee for Science and Technology. Tri is the scientist in charge of scientific relations with western countries. Tri admits that discrimination against intellectuals is a complicated social problem. "We have no difficulties with intellectuals in the North" he says, "but in the South there are two problems". According to Tri there is a class of intellectuals simply not prepared to work and another section anxious to return to their jobs but who are underemployed. "The first class" says Tri, "had a high standard of living under the previous administration and we are simply unable to maintain them in the standard to which they were accustomed so they are not willing to work for us. The other group of intellectuals including most of the scientists do want to share difficulties. In the South this is a period of transition. It is not a problem of freedom or discrimination; if people want to work they can."

Vietnam is still an underdeveloped country and as such cannot invest funds in many fields of science, Tri insists. "But our government" he continues, "tries to create facilities to enable scientists to work. We do not have enough rice to feed people yet we have built a new scientific centre—

the Vietnam Scientific Research Centre in Hanoi. Admittedly it is not well equipped, but we are trying hard for we think science is so important."

As for the universities in the south, all are open, says Tri. He adds that most of the research institutes have resumed working, and the scientists are being paid on a similar scale to those in the North. But salaries were not the only issue. According to some scientists, research institutes in the south did have a difficult period following the fall of Saigon in April 1975. In many cases 'political cadres' were appointed as temporary supervisors and scientists were under an 'air of suspicion'. However, with the gradual lessening of tension in Vietnam and the government's encouragement of a policy of 'reconciliation' this no longer seems to be an issue of great concern to scientists.



After the war: rebuilding begins

Prior to 1975 the majority of university graduates in South Vietnam were students of the social sciences or the arts. This is a pattern of education which has crippled the development plans of many third world countries. It is a state of affairs in Vietnam which will be changed. According to government sources much greater emphasis will now be placed on the teaching of science and engineering in the universities of the south.

Testimony to French presence

It would be wrong to discuss biology in Vietnam without referring to the National Institute of Hygiene and Epidemiology (IHE) in Hanoi. Standing in beautiful gardens the institute buildings are a lasting testimony to the French presence in Indochina. Buildings were designed for the comfort of French administrators. As such, they are ideally suited to house one of Vietnam's most impressive institutes.

When the IHE's function was explained by the Director Dr Hoang Thuy Nguyen it was easy to see why it was so well equipped. With the help of three sister institutes in the south in Saigon, Dalat and Nha Trang the IHE can supply enough freeze-dried

vaccines to meet the needs of the whole country. According to Nguyen they produce vaccines against "smallpox, tuberculosis, cholera, tetanus, diphtheria, whooping cough, leprosy, polio, rabies, encephalitis B and measles."

Nguyen's deputy Dr Trach has been involved with vaccine culture for many years. He says that "during the war we had a shortage of the vaccines for diphtheria and tetanus toxins, but for the others we had enough for the whole population". In the war years some sections of the institute were evacuated to the forests where according to Trach, vaccines were produced in equipment on a "smaller" scale. "This was much more expensive" says Trach "but we consider mass vaccination programmes to be one of the best methods of preventive medicine".

There is some doubt in Western medical circles about the usefulness of cholera vaccine as a preventive measure. Trach acknowledges this doubt but insists that the Vietnamese have found that cholera vaccination followed by booster doses every six months is an effective form of prevention. He adds that "improvement of hygiene" is obviously the best method of combating cholera, but that this takes "time and money". Immunisation is a way of doing this more cheaply as an interim measure.

With hygiene an important component of the IHE's work it is hardly surprising that it has pioneered a cheap, efficient latrine which enjoys widespread use in the countryside and is seen by many international aid agencies as ideal for many developing countries. It is simple in design, has separate channels for the collection of urine and faeces and makes use of a double septic tank. When the faeces tank is full, it is sealed to permit anaerobic degradation of bacteria after which the contents provide an important source of fertiliser. According to Nguyen they use the destruction of the "poliomyelitis virus as an index of measurement to determine how long the tanks should be sealed".

The IHE receives considerable financial support from the United Nations Children's Emergency Fund and for that reason it is one of the best endowed institutes in Vietnam. But the IHE, like many other research institutes in the country is really only at the beginning of its scientific development. Vietnam's scientists face tremendous obstacles before they can feel truly confident about the future. But given better harvests, the renowned resourcefulness of the Vietnamese, the assistance of the world scientific community, Vietnam will soon be making her own unique contribution to scientific knowledge. □

correspondence

Allegations against Indian research unit refuted

SIR,—It was regrettable that Robert Walgate's report of the Pugwash workshop in Delhi (2 March, page 8) gave some credence to the allegations made against the Research Unit on Genetic Control of Mosquitoes run in New Delhi by the WHO and Indian Council for Medical Research. The allegations have already been comprehensively refuted (*Nature*, 256, 353-357; 257, 175; 258, 102; *New Scientist*, 69, 88-89; 69, 528; *WHO Chronicle*, 30, 131-139). May we repeat that the unit's research had no connection with biological warfare; the releases made were of male mosquitoes which do not bite and therefore could not transmit disease. The research was principally on the vector of filariasis, a deforming disease which afflicts eight million people in India. At the time that the unit started its work this was certainly a more prevalent disease in India than malaria. Several months before the storm of press criticism broke the unit's joint Technical Planning and Review Group decided to increase the emphasis on *Anopheles* work in view of the clear signs of resurgence of malaria in India which were then becoming apparent. Field trials using chemosterilants have been conducted in the United States, notably the USDA Cotton Boll Weevil project (*Bull. Ent. Soc. Amer.*, 19, 218-221). The Indian and foreign scientists in the unit were well aware of the need for precautions in the use of chemosterilants: thorough washing of pupae after treatment was a routine procedure and this ensured that the residues in adult *Culex fatigans* were so low as to be undetectable by gas liquid chromatography (*Bull. Wld. Hlth Org.* 47, 675).

In one sense the falsity of the allegations is less important than the question why they were made and believed by many. One should recall that in the era of Watergate it was fashionable for journalists to 'discover' fiendish CIA-related plots. Unfortunately the Delhi journalists did not take the same care as Woodward and Bernstein to check the facts, the coherence of their story, or the credibility of their sources. Every claim made by the journalists was eagerly believed, and indeed embellished, by the Indian Parliamentary Public Accounts Committee (PAC), whose attitude of mind is illustrated by the fact that they published two sensational 200-page reports about the unit without taking evidence from any of its staff members or visiting the unit, which was situated in the same city as themselves.

The unit was highly productive of scientific data: 88 papers have so far been published on its work, predominantly under Indian authorship (which disposes of the allegation by the PAC that the work was conducted in "total secrecy" and indicates that it is certainly not true of this unit that "The third world scientists involved are left

untrained, and when the visiting team withdraws it takes its data with it"). The unit's fate and the Indian government's present attitude to scientific collaboration is indeed a severe setback to hopes for the application of modern biology to the solution of some of the problems of disease and hunger in the third world. The allegations could and should have been refuted as soon as they were made. The only lesson for the future is that administering authorities must face up to a prolonged and concerted campaign of this kind and not keep quiet and hope that it will all blow over.

C. F. CURTIS
Ross Institute of Tropical Hygiene,
London, UK

R. C. VON BORSTEL
National Institute for Medical Research,
Mill Hill, London, UK

Nature and nurture

SIR,—Many of the letters (6 April, page 490, 13 April, page 576) in response to mine (16 March, page 204) were wholly as expected. Evidently repeated exposures of the lack of scientific value of the arguments (Schwartz and Schwartz, *Nature* 248, 84, 1974; 255, 420, 1975) are brushed aside. What, however, did astonish me was the eagerness with which people ascribed the DDR's sporting successes to their selection and training of promising youngsters. Of course this is well known, but precisely the same happens everywhere (though perhaps with some variations of emphasis) in the intellectual and academic spheres. But in both sport and academic achievement, this factor is just part of the environment. Thus my inference is strengthened, not weakened, by this point.

HERMANN BONDI
Department of Energy,
London, UK

Statistician's plight

SIR,—I have been asked to draw the attention of scientific colleagues to the plight of Argentinian statistician Carlos Noriega, whose whereabouts are unknown following his arrest in February 1977. A group of statisticians working in the United Nations is requesting help in obtaining information. Readers of *Nature* may wish to petition the Argentinian government on his behalf.

H. P. WYNN
Royal Statistical Society,
London, UK

Methyl mercury and safety

SIR,—Methyl mercuric hydroxide has recently been employed in agarose gel electrophoresis of nucleic acids because it is the best available denaturing agent¹⁻³. We measured release of methyl mercury from agarose gels during preparation, electrophoresis and when localised heating was induced by cutting the gel. We found that toxic to lethal levels of methyl mercury can be released into the laboratory environment. Unless

full precautions are taken Minimata disease will probably appear among the operators of the technique.

For these experiments we used the buffer system described by Bailey and Davidson⁴ and an open horizontal gel⁴. This allowed us to study methyl mercury release from the exposed gel surface. Methyl mercury was determined using a Pharmacia mercury detector.

Our experiments reveal three points during electrophoresis when health could be endangered. The first is during addition of methyl mercury to the molten gel (2.3 mg m⁻³ released) and during casting of a gel (1.1 mg m⁻³ released). The second is during the first 10 min of current flow (0.19 mg m⁻³ released). The third is when localised heating results from breaks or imperfections in the gel (4 to 8 mg m⁻³ released). Methyl mercury release is negligible during all other phases of the operation.

70-90% of organic mercury inhaled is delivered to the bloodstream⁵. A body burden of 25 mg of methyl mercury is generally the level at which noticeable symptoms appear. These symptoms include permanent central nervous system damage. Death generally follows accumulations greater than 200 mg, but as in the case of the early symptoms there can be a delay of weeks following exposure before the damage is fully expressed⁶.

A single exposure would not exceed 0.4 mg ingested methyl mercury under the most extreme conditions. Unfortunately, the half-life for methyl-mercury retention in the human body is more than 70 days. Thus, large numbers of experiments during a one or two month period could lead to body accumulations more than sufficient to produce symptoms of Minimata disease.

We suggest the following precautions:

- All work with methyl mercury should be confined to a well-vented laboratory-hood equipped with back-up power in case of power failure.
- Handling and disposal of waste materials should be conducted with the care given volatile radioactive compounds.
- Operators of the technique should be tested regularly for blood mercury levels.
- Pregnant women should not work in laboratory rooms where methyl mercury gels are prepared, as birth defects may be caused by low levels of the compound.
- Journal editors should make an effort to ensure that all papers describing work with toxic materials refer to the materials' abilities to pollute the laboratory environment.

J. E. CUMMINS
B. E. NESBITT
University of Western Ontario,
London, Ontario, Canada.

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Interferon, dsRNA and the pleiotropic effector

In the article '10 nm filaments' (*News and Views* **272**, 577; 1978) line 16 paragraph 2 on page 577 should read '. . . the brain preparation contains a mixture of immunologically distinct neurofilament and glial filament chains, both of molecular weight ~ 50 K . . . '.

that a very few molecules, perhaps only one per cell, induce at least the two enzyme systems described above, and that these systems are both switched on by dsRNA, and that they act in their different ways to inhibit protein synthesis. Is it really true that dsRNA is an intracellular sign of virus infection? The cell's response to dsRNA is astonishingly sensitive—one molecule of dsRNA the size of a reovirus genomic segment should be enough to turn off protein synthesis—but one wonders whether dsRNA truly appears in the cytoplasm of all virus-infected cells. Do cells have some other way of recognising that they have been infected? And if so, does this signal operate the same switches as dsRNA? The puzzle is to discover what all viruses have in common that the cell distinguishes as abnormal, for it must be emphasised that interferon has very little effect on cell growth; the trigger of viral infection is crucial. I can only think that all viruses have to enter the cell, that they may let in foreign substances from the external environment, or disturb the membrane in a characteristic way. If so, the molecular basis is unknown, and for the moment we had perhaps better think about dsRNA.

Finally, the question of specificity. A cell is exposed to interferon, and makes these defensive enzymes. Along comes a virus, makes some dsRNA and thus announces its arrival. The cell responds by turning off initiation and destroying mRNA. Why doesn't the cell die? In some cases it does (which is a perfectly good way of preventing viral replication), but this is not the invariable outcome. There are even reports that host protein synthesis continues normally in some systems, although the virus fails to make any of its own proteins; at present this is impossible to explain. One might suppose that the endonuclease activated by two-five A is a sort of restriction enzyme, highly specific for viral mRNA, but it is not (it degrades globin mRNA, for example), and nor is this a plausible idea, for viruses would surely lose their nuclease-sensitive sites by natural selection. David Metz (*Cell* 6, 429; 1975) made the ingenious suggestion that the very features (whatever they may be) that give viral messages a selective advantage (if they do) in a normal infection may be recognised by the interferon-treated cell as the basis for selective rejection. But if this were so, why are not cells in a permanently anti-viral state? And would not such features have also been eliminated by selection? This is an area where much more work is needed, and preferably not of the comparing globin mRNA with EMC RNA genre; because these two commonly used mRNAs differ by

a factor of ten in their size, it is extremely hard to compare their relative template activities in a meaningful way. (Though notice how picornaviruses are adapted to making proteins under conditions of reduced initiation rates; they make a dozen or so proteins from each initiation event). Still, it seems possible to me that in many systems these antiviral defences may operate by shutting off protein synthesis, destroying all cytoplasmic mRNA, cellular and viral alike, wait-

ing until the virus is dead, and then turning protein synthesis back on and making fresh mRNA. We need to know much more about the specificity of the nuclease, two-five A and the protein kinase, and conversely how they are all turned off as well as on before we understand even this part of the system. But thanks to Ian Kerr, Peter Lengyel, Michel Revel and their colleagues for making it all so much clearer so far; keep up the good work! □

Another surprise from Gargamelle

from David J. Miller

NEUTRINOS can interact with the nuclei of ordinary matter or they can interact with the atomic electrons. The key to the whole of our modern understanding of the weak interaction was the observation, first made with the Gargamelle bubble chamber at CERN, Geneva, that when an incoming neutrino collides with a nucleus it can rebound without gaining an electric charge (Hasert *et al. Phys. Lett.* 46B, 138; 1973) see also *News and Views* 245, 119; 1973). This neutral-current (NC) process is in contrast with the charged-current (CC) processes in which muon—or electron—neutrinos collide with nuclei and are transformed into charged muons or electrons. The CC neutrino processes involving a nuclear target (for example diagram *a*) are directly related to other interactions between hadrons (strongly interacting particles) and leptons (electrons, muons and their neutrinos) such as nuclear β -decay (see *b*), hyperon decays and the decays of π and K-mesons. Until recently only one purely leptonic weak interaction process had been available for study, the muon decay $\mu^- \rightarrow e^- \nu_\mu \bar{\nu}_e$ (see *c*). Here two charged leptonic currents interact, one of them turning a muon into a mu-neutrino, the other generating an electron and its antineutrino. In the diagrams the currents are represented by continuous lines, labelled with the names of the particles. The dotted lines labelled $W^\pm$ or W^- represent a virtual charged vector boson which is supposed to carry the weak interaction between the two charged currents. Time runs from left to right and arrows going backwards in time describe antiparticles.

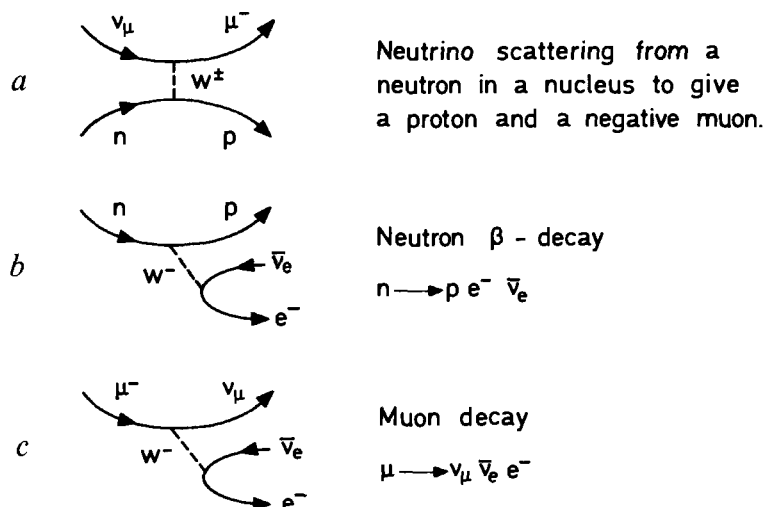
Since 1973, on the basis of the observations in Gargamelle and elsewhere of hadronic NC neutrino inter-

actions, a selfconsistent and unified theory of both the weak and the electromagnetic interactions has been sought. The simplest candidate was the Weinberg-Salam model in which a neutral vector boson Z^0 (diagram *e*) transmitted the interaction between neutral currents (see Leader & Williams *Nature* 257, 93; 1975). The Z^0 should, in a unified theory, be related to the photon (γ in diagram *d*) which transmits the electromagnetic interaction between two electric currents. Once the relative strengths of NC and CC interactions of neutrinos with nuclei had been measured it was possible to estimate a parameter called the Weinberg angle which fixed the relationship between the photon and the Z^0 . Predictions could then be made of other types of NC interaction such as neutrino-electron elastic scattering or parity non-conserving effects in atomic processes (see *f*). Already in 1977 it was clear that the atomic effects were not being seen at the level predicted by the Weinberg-Salam model (see Feinberg *Nature* 271, 509; 1978). The new Gargamelle result also suggests a clear violation of the model.

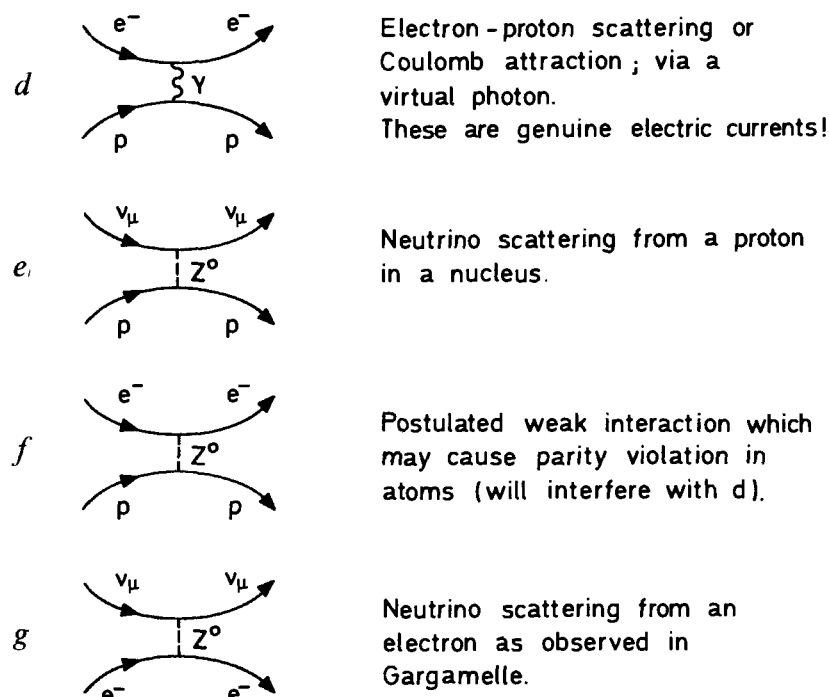
During 1977, before the Beam Dump Experiment (see *News and Views* 272, 205; 1978), Gargamelle was exposed to the neutrino beam at the CERN SPS. The film was analysed by a collaboration of physicists from Bari, CERN, Ecole Polytechnique, Milan and Orsay. In a report to be presented at a conference in Purdue (also submitted to *Physics Letters*) they describe how they scanned for examples of the process in diagram *g*, the elastic scattering of muon neutrinos from atomic electrons. This is the NC analogue of μ decay. The Weinberg-Salam prediction was that in this experiment, containing 22,000 CC hadronic interactions, only 1.6 elastic neutron-electron events should be

David J. Miller is in the Department of Physics and Astronomy in University College, London.

EXAMPLES OF CHARGED-CURRENT WEAK INTERACTIONS



EXAMPLES OF NEUTRAL - CURRENT INTERACTIONS (Electromagnetic or Weak)



expected. But the Gargamelle collaboration sees 12 events, with an estimated background of about one fifth of an event. Previous experiments have looked for this process but none until now has had sufficient sensitivity to obtain a clear result.

There are several other current bubble chamber experiments (both at CERN and at Fermilab) which should be able to check this, so we shall soon see if the 12 events in Gargamelle are merely a statistical fluctuation. It will also be very interesting to see how

the antineutrino-electron scattering behaves.

It was expected that the Weinberg-Salam model would prove inadequate as a complete theory of weak and electromagnetic interactions. At last year's Hamburg conference (see *News and Views* 269, 86; 1977) Steven Weinberg himself referred to some of the benefits that would come from a more complicated model of the weak interaction. Room is needed for a theory of CP violation in K^0 decays, for the new τ lepton and for the interactions of the new quarks which may be associated with the upsilon particle. But it is unexpected for the Weinberg-Salam model to fail in its prediction of the rate for the simplest of all neutral current processes. Perhaps this unexpected result will make it possible to converge more rapidly upon a believable new theory out of the vast and ingenious range of models on offer. $\square$

Regulating the immune system

from Elizabeth Simpson

THE immune system discriminates between self and non-self so that normally, immune responses are only mounted against non-self components. To account for the failure to attack self, Burnet (*The Clonal Selection Theory of Acquired Immunity*, Cambridge University Press, 1959) proposed the clonal selection theory whereby all clones of lymphocytes bearing receptors against self determinants would be eliminated. However, any clones of cells reacting against foreign non-self determinants (for example, bacterial, viral or transplantation antigens) and undergoing cell division and differentiation to mount a protective response for eliminating such undesirable antigens could themselves, if not subject to further control, pose a threat by continued multiplication—becoming a tumour.

Two basic theories have been put forward to account for the control of the immune response normally seen: one, proposed originally by Gershon (for review see Gershon, *Contemporary Topics in Immunobiology*, 3, 1; 1973) was that all immune responses were under the control of the T cell subdivision of lymphocytes, and that in addition to the 'helper' effects of T cells (Mitchison *Eur. J. Immun.* 1, 18;

Elizabeth Simpson works at the Clinical Research Centre, Harrow.

1971), suppressor effects were also mediated by T cells, acting whenever the magnitude of the response was high. When first proposed, this notion was not considered very respectable, but since then it has been clothed by more and more evidence, from a wide variety of immunological situations, that an identifiable and separable subclass of suppressor T cells exists (Jandinski *et al.* *J. exp. Med.* **143**, 1382; 1976; Beverley *et al.* *Nature* **262**, 495; 1976). The other theory proposed to account for control of proliferation of cells of the immune system was that of Jerne (in *Cellular Selection and Regulation in the Immune Response*, (ed. Edelman, G. M.) 39 (North Holland Publishing Company, 1974)). He saw the immune system as a network of antibodies and receptors that recognise and are recognised by other antibody molecules and lymphocytes: thus, when, for example, an antibody response is made to an extrinsic antigen, the idiotype (the antigenic determinants unique to that antibody by virtue of its specificity for that particular extrinsic antigen) of that antibody will in turn act as an antigen, and elicit an anti-idiotype response, and this will limit the extent of the initial antibody response. In turn, the idiotypic determinants of the anti-idiotype response will stimulate an anti-anti-idiotype response and so on.

There is certainly evidence for control of antibody responses by responses directed against idiotypic determinants and against allotypic determinants on immunoglobulin molecules (for example, Rowley *et al.* *Science*, **181**, 1133; 1973; Herzenberg *et al.* *J. exp. Med.*, **144**, 330; 1976). The effector cell is certainly not always a B cell (it is a T cell in allotype suppression) and the target cell may be either the T helper cell, or the antibody-producing B cell. Although Jerne's theory does not exclude the participation of T cells in his regulatory network, it does not envisage a particular subclass of suppressor T cells as postulated by Gershon. However, knowledge that T and B cells share idiotypic determinants (Eichmann & Rajewsky *Eur. J. Immun.*, **5**, 661; 1975; Binz & Wigzell *Cold Spring Harbor Symp. quant. Biol.* **41**; 1977) provide a basis for considering that suppressor T cells may operate within the anti-idiotype network.

The paper by Cooke *et al.* published in this issue of *Nature* (page 154) brings together many of these ideas, in an experimental model (auto-immune haemolytic anaemia) which explores the role of regulation in auto-immune responses. It suggests that even when 'forbidden clones' of lymphocytes emerge (by immunisation

of mice with rat red cells, which share some determinants with mouse red cells, but also have other, foreign determinants, which presumably stimulate the response of T helper cells in the mouse) and autoantibody against self red cells is made, suppressor mechanisms are set in motion to limit the production of autoantibody. This suppressor mechanism has characteristics of an immune response, since it shows both memory and specificity. Thus in mice immunised several times with rat red blood cells the time during which autoantibody can be detected is decreased in comparison with mice receiving one dose of rat red blood cells, and the concomitant anti-rat-specific red cell antibody response is not affected. Furthermore rapid turn-off of the autoantibody response can be transferred to naive syngeneic recipients by T cells from mice that have received one or more immunisations with rat red blood cells. This implicates

suppressor T cells as the effector population. The target of their action may be either the idiotype of the B cells producing autoantibody, or that of the helper T cells presumably raised to a carrier determinant on the rat red cells.

The implications of this work are that in addition to the regulation of responses to extrinsic antigens, the immune system can regulate responses to 'self' antigens and that this regulation is mediated by suppressor T cells. 'Forbidden clones' of self-reactive lymphocytes may not just be eliminated during ontogeny, in the thymus, as originally proposed, but may also be removed in the periphery by this T suppressor mechanism. The appearance of autoimmune disease may be due to a defect in suppressor T cells, as has been suggested for strains of mice with a high incidence of autoimmunity (de Heer & Edgington *J. Immun.* **113**, 1184; 1974). □

Spinning comets and planets

from David W. Hughes

ROTATION is a common property of all Solar System objects. However knowing that everything is spinning is a far cry from being able to measure the spin rate accurately. A brief perusal of old astronomy books shows that today's observed planetary spin rates differ considerably from those measured a decade or two ago. In R. S. Ball's *Popular Guide to the Heavens* (1905) Mercury, Venus, Uranus and Neptune were all quoted as having unknown spin rates. By the 1950s these were given as 88 d, 30 d, 10.7 h and 15.8 h. Today the values are 58.6 d, 243.1 d, 17 to 22 h and about 23 h.

The problems encountered when trying to explain these values are not insignificant. A. W. Harris (*Icarus* **31**, 168; 1977) concludes that something more is required than just the steady growth of planets and increase in angular momentum produced by the accretion of planetismals arriving at very low encounter velocities. The variation from one planet to the next of the angle of inclination of the spin axis to the ecliptic plane is usually thought to be caused by the random dispersion of the rotation and velocity vectors of the incident planetismals. Still one has to explain why Venus

and Uranus have retrograde spins.

Of course the spin rate might not be constant as a function of time. S. S. Kumar (*Astrophys. and Space Sci.* **51**, 235; 1977) suggests that the slow rotations of Mercury and Venus may be connected with the absence of natural satellites around them. At formation Kumar suggests they had rotation periods similar to the present periods of the other members of the Solar System. Exchange of angular momentum between a receding satellite and a planet with a decelerating spin rate eventually placed the satellite into such a large orbit that the Sun's gravitational influence exceeded that of the planet's. The satellite then escaped into a heliocentric orbit. To reduce the spin rate of Mercury and Venus to their present values obviously requires a large satellite; our Moon has only changed Earth's spin rate by a factor of around two during its lifetime. It is not beyond the bounds of possibility that the missing satellite of Venus was none other than Mercury itself.

The end product of these tidal interactions is an equating of the spin period of the planet and the satellite to the orbital period of the satellite. The period of Venus is only four times longer than that of Mercury. However the tidal action of the Sun on Mercury and Venus could also have reduced their

David W. Hughes is a lecturer in the Department of Physics, University of Sheffield.

spin speed considerably. The presence of liquid water on their surfaces would have helped but is not essential, viscous friction by bodily tides being sufficient.

As all things spin one would expect comets to spin too. This of course presupposes that comets have a large majority of their mass concentrated in a dirty snowball nucleus and not in a vast dust swarm of tiny particles (see *News and Views* 270, 558; 1977). Spinning nuclei have been used to explain the non-gravitational motion of comets. Comet Encke has a period which is decreasing by 2.5 hours per orbital revolution. Comet Halley on the other hand persists in arriving late at perihelion by an average 4.1 days over the past 11 apparitions. Solar radiation causes the ice in the nucleus to evaporate. Molecules are ejected on the sunward side, generating a jet reaction. If the comet rotates the jet is displaced from the radial direction exerting a force on the nucleus which speeds it up if the spin is in the opposite direction to the orbital motion of the comet around the Sun. If the comet is spinning in the same sense as the planets the jet force slows down the comet, increasing its period and the semi-major axis of its orbit. As comets seem to have spin axes which are randomly orientated in space half seem to be accelerated and half decelerated. Unfortunately the acceleration or deceleration depends on the spin axis direction, spin period and the gas ejection rate. So measurement of this acceleration does not yield an unequivocal value for the spin rate.

Fred Whipple of the Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts has been able to measure this spin rate using another technique. This is reported on page 134 of this issue of *Nature*. Whipple considers observations of Donati's comet which was discovered on 2 June 1858 by Giovanni B. Donati, a famous Italian astronomer and pioneer spectroscopist and observed for about 10 months. It has the reputation of being one of the most beautiful comets seen and had a most pronounced curved main tail. The period was thought to be around 2,000 years and it has been suggested that Donati is identical to the great comet recorded by the Roman writer Seneca in 146 BC.

One of the more unusual characteristics of Comet Donati was that its coma contained a series of haloes. These have been interpreted as expanding shells of material which seemed to be emitted with considerable violence from the central nuclear region. Haloes have also been seen in Comets Coggia (1974 III), Morehouse (1908 III) and Schwassmann-Wachmann I (1925 II). In Donati the haloes

Methanogenic bacteria

Dr J. A. Steitz finds it difficult to accept¹ our claim that methanogenic bacteria are representative of a group of organisms, the archaeobacteria, that in a genealogical sense bear no more relationship to the typical bacteria than they do to (the "cytoplasmic aspect" of) the eukaryotes²⁻⁴. Citing a functionally defined pentanucleotide segment occurring very near the 3'-terminus of all bacterial 16S rRNAs⁵⁻⁸, but not found in eukaryotic 18S rRNAs⁹⁻¹¹, she concludes that "a high degree of functional relatedness between methanogenic and other 16S rRNAs" exists¹, implying thereby that archaeobacteria are specifically related to all other bacteria, and do not, therefore, represent a separate line of descent, distinct from the typical bacterial and the 'eukaryotic' lines^{3,4}.

What Steitz has done is to base a genealogical conclusion upon a single (simple) character—a procedure eschewed by systematists. Given three major lines of descent from a common ancestor, one would indeed expect to find numerous examples in which a single character is found in only two of the three lines. For this reason, classification should be, and is, based on multiple characters and/or composite characters such as cell wall¹²⁻¹³ or ribosomal RNA¹⁴ (in its entirety).

It is perhaps more convincing to give some specific examples: As pointed out originally, the usual tRNA common arm sequence, GT ψ CG, is not found in archaeobacteria³, which might then suggest a "functional relatedness" between eukaryotic and typical bacterial tRNAs. Yet the initiator tRNA in (at least one of the) archaeobacteria has characteristics that group it specifically with its eukaryotic counterparts¹⁵. In terms of lipids, typical bacteria resemble eukaryotes rather than archaeobacteria¹⁶⁻¹⁸. In terms of rRNA sizes and Steitz's criterion, typical bacteria and archaeobacteria group together^{3,19}. (A synopsis of archaeobacteria and their characteristics will appear shortly²⁰.)

Overall it seems that the number of molecular characteristics the archaeobacteria share specifically with

typical bacteria is no greater than the number they share with eukaryotic cells. Moreover, archaeobacteria possess striking features not found in either typical bacteria or eukaryotes.

We see no reason at present to rescind the conclusion that archaeobacteria and typical bacteria represent separate lines of descent not related to one another specifically. Quite the opposite. All evidence accumulated pursuant to our initial discovery has tended to confirm and strengthen the case for the genealogical uniqueness of archaeobacteria (see ref. 20 now in press).

C. R. WOESE

Department of Genetics and Development,
University of Illinois at Urbana-Champaign,
Illinois 61801

G. E. FOX

Department of Biophysical Sciences,
University of Houston,
Houston,
Texas 77004

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were regular and sharp and Whipple assumes that the source of each halo is a massive mass ejection from a single active region on the cometary nucleus. This occurs every time that region becomes exposed to solar radiation, ejections being separated in

time by the cometary rotation period.

The ejected mass is moving away from the nucleus at a velocity which only depends on the distance between the comet and the Sun. Knowing the Earth-comet distance Whipple then calculates the spatial separation

between the halo and the nucleus. Dividing this by their velocity gives the time of halo emission (its zero date). The average difference between the zero dates of successive haloes was found to be 4.6 hours, this being the rotation period of the nucleus.

Whipple stresses quite rightly that this regularity of cometary behaviour is very strong evidence for the existence of a discrete central nucleus. In 1863 J. F. J. Schmidt analysed his own observations of Comet Donati and obtained a period of 4.3 h. Unfortunately Schmidt did not take the next intuitive step and interpret his result in terms of a spinning concentrated nucleus.

The danger point for equatorial ejection occurs when the rotational surface velocity of the body is equal to its escape velocity. This occurs when the product of the period T in hours and square root of the density ρ in g cm^{-3} is given by

$$T\rho^{1/2} \approx 1.32$$

Hughes (*J. Br. astr. Ass.* **84**, 272; 1974) found that the icy comet nucleus had a density of around 1.14 g cm^{-3} so it would be distinctly unstable if it were spinning with a period of less than 1.2 hours.

The period of around 4.6 h puts Comet Donati in the same ball park as asteroids (Psyche 4.3 h, Vesta 5.3 h, Eunomia 6.1 h, Eros 5.3 h, Ceres 9.1 h). But it must be remembered that asymmetrical gas ejection can vary the cometary spin period. They can be spun-up until they become unstable and split, or they can spin down, becoming more stable and less prone to orbital changes produced by non-gravitational effects. $\square$

Pion production in heavy-ion collisions

from P. E. Hodgson

LAST year McNulty *et al.* (*Phys. Rev. Lett.* **38**, 1519; 1977) measured the frequency of pion emission from the interactions of 280 MeV per nucleon neon nuclei with nuclei in G5 nuclear emulsion and found that it was very much higher than the rate calculated by Bertsch from the independent particle model. This suggested that collective effects, perhaps associated with pion condensation, greatly enhance the frequency of pion production (see *News and Views* **268**, 586; 1977).

It has now been found by Lindstrom *et al.* at Berkeley (*Phys. Rev. Lett.* **40**, 93; 1978) that these measurements are probably in error, due to

ARC unit closes

by Mary Lindley

A SILVER jubilee and a death knell were marked simultaneously on 25 April with a botanical reunion at Wye College in Kent. After 25 years of major contributions to plant science, the Agricultural Research Council's Unit of Plant Growth Substances and Systemic Fungicides is being closed on the retirement of its director R. L. Wain. He is departing to the peaceful pastures of an emeritus professorship at the University of Kent, and his staff, friends and colleagues past and present paid tribute to him with a symposium.

The unit has been in the forefront of research into the properties and application of naturally occurring plant substances. As Wain reminded the visitors, those substances have included abscisic acid, so named for its role in leaf abscission; xanthoxin, a hormone inhibitor and 'a Wye product through and through'; and the fungicide wyerone. Wain's own special genius has been for chemical synthesis, according to L. Fowden (Rothamsted Experimental Station). He described Wain as a great manipulator of the carbon-halogen bond, who has produced compounds that have since assumed great value as, for example, herbicides and specific growth antagonists. The value of such achievements was endorsed by Sir William Henderson (Agricultural Research Council, London) whose search of the council's archives had revealed that the funds voted for the unit's first year of operation in 1953 amounted to a munificent £4,650.

The staff of the unit will be taking their expertise to four of the council's institutes—Rothamsted Experimental Station, Long Ashton Research



Professor R. L. Wain (left) and Sir William Henderson.

Station, the Weed Research Organization and the Letcombe Laboratory, all concerned in some way with the promotion or inhibition of plant growth. Past and future interests of the members of the unit were reflected in the contributions to the symposium. In particular there was the affection of T. A. Mansfield (University of Lancaster) for stomata—those remarkable sense organs, which, in contrast to popular belief, do not merely open by day and close by night. He would like to see plant hormones used to manipulate them and thus influence photosynthetic efficiency. F. Wightman (Carleton University, Ottawa) is adding new vigour to plant studies with the news that phenylacetic acid seems to be one of a new group of naturally occurring plant growth promoters. Whatever future work may reveal about the control and manipulation of plant growth, those from Wye will inevitably be in the thick of it.

Mary Lindley is an Assistant Editor of *Nature*.

the misidentification of proton tracks as pion tracks. It is not easy to distinguish between the tracks of relativistic protons and pions in nuclear emulsions, because both have ionisation close to the minimum value. McNulty *et al.* used multiple scattering measurements to do this, and Lindstrom *et al.* conclude that these must have been in error.

In their work, Lindstrom *et al.* examined a similar stack of emulsion exposed to neon nuclei of about the same energy, and looked for pions coming to rest in the emulsion. This is a much more definite measurement

because the tracks of pions and protons can easily be distinguished near the ends of their ranges. In an area of emulsion that should, if the previously reported production rate were accurate, contain the tracks of about 50 stopping pions they did not find any. This shows that the rate of pion production is less than 0.06 of that found by McNulty *et al.* and is thus not inconsistent with the predictions of the independent particle model.

P. E. Hodgson is a Lecturer in Nuclear Physics at the University of Oxford.

articles

Solar modulation of atmospheric electrification and possible implications for the Sun–weather relationship

Ralph Markson

Massachusetts Institute of Technology, Department of Aeronautics and Astronautics, Measurement Systems Laboratory, Building W-91, Cambridge, Massachusetts 02139

Atmospheric electrical processes may help explain statistical findings indicating that variable solar activity, such as solar flares or the Earth's position in the extended solar magnetic field, affects the weather. An atmospheric electrical mechanism bypasses difficulties associated with solar heating mechanisms. Understanding Sun–weather relationships offers a basis for long range weather forecasting.

THE possibility that solar variability affects the Earth's weather has received much attention recently because of the record cold 1976–77 winter and severe droughts in parts of the US and Europe. These came at a minimum in the 11-yr sunspot cycle which is also the predicted time for drought according to the 22-yr double sunspot cycle¹. This cycle has been correlated with a long-term US recurrence pattern including during the last two cycles the 'dust bowl' of the mid-1930s and the drought of the mid-1950s as well as similar drought cycles in other parts of the world². Parker³ and Eddy⁴ indicated that solar variability can cease for extended periods and that these intervals coincide with the extremes of low temperature periods on Earth. Between 1645 and 1715 there were almost no sunspots and similar situations apparently occurred during earlier epochs. This makes us revise our notion that the Sun is a well behaved and predictable environmental feature routinely following a roughly 11-yr sunspot cycle. If climate is influenced by variable solar activity, we are faced with the difficult problem of explaining the cause. In this context it is important to recognise that 'climate' is an integrated long duration average weather condition composed of many individual short-term meteorological events.

There are hundreds of statistical correlations between solar activity and weather⁵, but scientists are generally sceptical because the driving force for the Earth's atmosphere, solar heating, is essentially constant. I explain here how solar activity should affect one basic property of the atmosphere, atmospheric electrical responses, even if it has no influence on cloud physical processes. Stratospheric ionisation and higher electric field intensities could, however, also enhance cloud electrification. Finally, speculations on how thunderstorm electrification might modulate rainfall and atmospheric circulations are mentioned. An outline of the Earth's atmospheric electrical global circuit is presented so that electrical processes which might be important in Sun–weather relationships can be considered.

Because of greatly increased understanding of solar, space and upper atmosphere physics, due primarily to satellite observations, we can now reconsider the possible solar influence on weather. Definition of the structure of the interplanetary magnetic field⁶, which organises the flux of solar and galactic radiation reaching the Earth's atmosphere, led us to use the time the Earth crosses solar magnetic sector boundaries as 'key days' in statistical studies of Sun–weather relationships⁷. This initial study suggested a variation of US thunderstorm activity as a function of the Earth's position in solar sectors. This solar sector approach produced many new findings of meteorological responses to solar variability. The most widely discussed result, possibly because of its high statistical reliability, was the response of atmospheric vorticity in the Northern Hemisphere^{8,9}. Vorticity characterises cyclonic atmospheric circulation which is associated with disturbed weather and cross-latitude air motions. Recent studies relate solar sector crossings to atmospheric electrical parameters—lightning frequency¹⁰ and thunderstorm frequency¹¹—as well as electric field variations in the Arctic^{12,13}, the Antarctic¹⁴, and mid-latitude mountain tops¹⁵. According to Hines¹⁶, introduction of the solar sector parameter opened a "new era in the study of Sun–weather correlations . . ." Hines and Halevy¹⁷ have examined the response of atmospheric vorticity to solar sectors and concluded that some physical mechanism must exist whereby variable solar activity influences weather.

Another solar controlled timing mark used is the occurrence of solar flares. An atmospheric electrical response in which electric fields^{18–20} and lightning occurrence^{19,21} increase shortly after solar flares have been found. On a longer time scale, high positive correlation between the 11-yr sunspot cycle and thunderstorm activity in England has been reported²². (Flares are the most significant solar phenomenon associated with sunspots. An explanation for these atmospheric electrical responses to solar activity is suggested here and may provide important clues to how the Sun's variations modulate weather.

That solar disturbances might modulate thunderstorm activity and atmospheric electricity has been postulated for over 100 years. Enough data have been collected to suggest that while such effects may occur, the problem is complex; direct and inverse correlations as well as a phase shift have been reported. (Some older studies are reviewed in refs 7 and 23.)

If thunderstorm frequency and/or intensity are altered, we expect additional meteorological consequences. Thunderstorms are one of the most energetic natural phenomena; an average storm releases energy of about 10^8 kW h, equivalent to

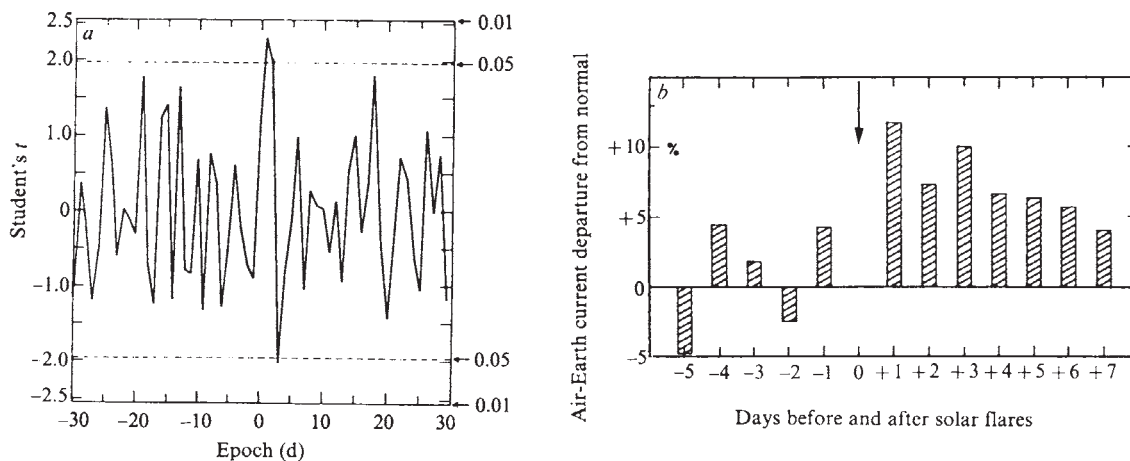


Fig. 1 *a*, Student's *t* values for 24-h changes in vorticity area index (expressed as departure from mean) before and after 94 large flares, 1955–69 (winter season, October–March). Arrows indicate labelled confidence limits. *b*, Average daily departure from normal of the fair-weather air–Earth current before and after solar flares.

10 Nagasaki type atomic bombs²⁴. Release of latent heat when water vapour condenses into rain is the major source of energy for driving atmospheric circulations²⁴.

Problem with heating mechanisms

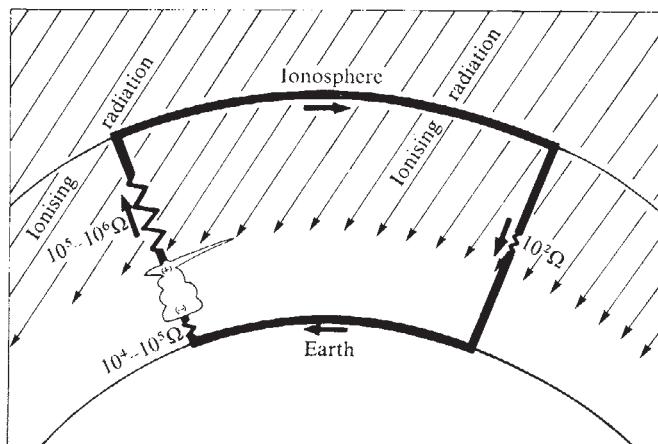
None of the physical mechanisms advanced to explain solar control of weather and have been generally accepted²⁵. Most consider processes which depend directly or indirectly on changes in solar heating. Here we suggest a different approach in which solar controlled variation of stratospheric ionisation explains atmospheric electrical variations and possibly other meteorological responses. An atmospheric electrical mechanism bypasses the difficulties inherent with heating mechanisms in several ways: (1) Energy: the major obstacle in explaining a solar influence on the Earth's atmosphere is that the solar constant variation is very small—within the 1% error of past measurements²⁶. The atmospheric electrical mechanism requires no solar energy to change the state of the Earth's electric field and stratospheric conductivity, while offering the possibility of releasing and redistributing large amounts of energy already present in the troposphere. (2) Coupling: while solar controlled variations of temperature occur in the upper atmosphere (thermosphere), this region is at best only very weakly linked to the lower atmosphere where meteorological responses to solar variability have been observed^{27,28}. With an atmospheric electrical mechanism it is unnecessary to couple energy

from the upper to the lower atmosphere as the entire electric field from ionosphere to Earth would be affected; in fact if the ionospheric potential varied the largest absolute variation in electric field intensity would occur near the ground where electric fields maximise. (3) Time delays: heating mechanisms are presumed to require at least several days before atmospheric dynamics would be affected²⁹. However, the vorticity area index maximises only one day after strong solar flares³⁰ (see Fig. 1*a*). Hence Roberts and Olson concluded that heating due to cirrus cloud formation cannot cause the rapid vorticity response to solar flares and they are, therefore, considering the possibilities of atmospheric electricity (personal communication and ref. 31). An atmospheric electrical mechanism offers the required rapid response; a change in ionospheric potential would rapidly affect electric field intensities all over the world³². Figure 1*b* shows¹⁸ that this occurs one day after solar flares.

Global circuit and influence of ionising radiation

To explain solar modulation of atmospheric electricity we first review the 'global circuit'^{33,34} shown in Fig. 2: the proposed mechanism functions within the framework of this circuit. According to this concept, worldwide thunderstorm activity is in effect a d.c. generator (vertical dipole) causing current to flow through the circuit and maintaining the Earth's electric field. If thunderstorms were to stop, the electrification of the atmosphere would decay with a time constant of about 15 min (ref. 32); it persists because thunderstorms occur somewhere at all times³⁵. Conduction currents can flow in the atmosphere because it is ionised. Atmospheric conductivity is maintained mostly by the constant isotropic flux of galactic cosmic radiation. It is augmented in the stratosphere at times of solar activity by solar corpuscular radiation and X rays from magnetospheric and auroral particle precipitation. In addition, near the Earth's surface over land, radioactive gasses and emanations from the ground account for about two thirds of the ionisation. Above the planetary boundary layer, conductivity increases essentially exponentially with altitude³⁶ partly because of the increase in cosmic radiation³⁷. An equipotential surface is considered to exist at about 60 km because, by this altitude, conductivity is so great that potential gradients are very small³⁸. The polar cap regions are exceptions where ionospheric electric generators can exist³⁹. This equipotential surface is called the 'ionosphere' when describing the global circuit; it is the outer shell of a spherical capacitor, the inner conductor being the Earth's surface. A leaky dielectric, the atmosphere, exists between the conductors. Above the 'thunderstorm generator'—the charging resistor—a conduction current flows between thundercloud

Fig. 2 The atmospheric electrical global circuit. Large arrows indicate flow of positive charge. Estimated resistances of circuit elements are given. The thunderstorm depicted represents the global electrical generator, that is, the totality of all global thunderstorms.



tops and the ionosphere. This charging current maintains the ionospheric potential at about +250 kV relative to the Earth^{40,41}. Ionospheric potential is an expression of the overall intensity of global atmospheric electrification; it can be measured by integrating aircraft^{42,43} or balloon⁴⁴ soundings of vertical potential gradient from the Earth's surface upwards. Over the fair-weather portions of the globe, in the 'return' portion of the circuit, a conduction current flows through the 'load resistor' part of the circuit: positive ions drift downwards and negative ions drift upwards. The circuit is completed between Earth and the thundercloud's base through combined hydrometeor precipitation, conduction (including corona), convection and lightning currents⁴⁵.

Figure 3 shows how estimates of resistances in the global circuit elements were derived. An order of magnitude approximation for the thunderstorm generator is 1,500 storms at 1 A per storm for a total current of 1,500 A (ref. 46). The tops of thunderclouds are at potentials of 10^8 – 10^9 V relative to Earth and ionosphere⁴⁷. This gives a resistance of 10^5 – 10^6 Ω for the charging resistor over the thunderstorm generator (all thunderstorms in parallel). The load resistor, in the return flow over most of the Earth's surface, is the ionospheric potential divided by the total current—about 165 Ω (ref. 48). Columnar resistances are given on the left side of Fig. 3. Columnar resistance is defined as $h_2 \int_{h_1} (1/\lambda) dh$ where λ is atmospheric conductivity and h is height. Its dimensions are Ωm^2 ; thus dividing by the horizontal cross-sectional area yields resistance between the top and bottom of an atmospheric column with that cross-sectional area between height (h_1) and height (h_2). Columnar resistance per unit height and cross-sectional area (specific resistance) decreases with altitude because of the increase in conductivity. As columnar resistance between the height of the top of the thunderstorm generator and ionosphere is about 10% of the columnar resistance from that level to Earth, the load resistor may be divided into two resistances of 15 Ω above that level [$R_{\text{world (high)}}$] and 150 Ω below it [$R_{\text{world (low)}}$]. The resistance between the thunderstorm generator and Earth would be twice that of the charging resistor over the thunderstorm except for the effect of point discharge ions⁴⁹. Because of strong electric fields under thunderclouds, pointed objects on the ground go into corona ionising the volume beneath the storms. It has been estimated that conductivity would be increased by three orders of magnitude in this region⁵⁰, but some measurements⁵¹ have recorded an increase of about one order of magnitude; here we assume that a 20-fold

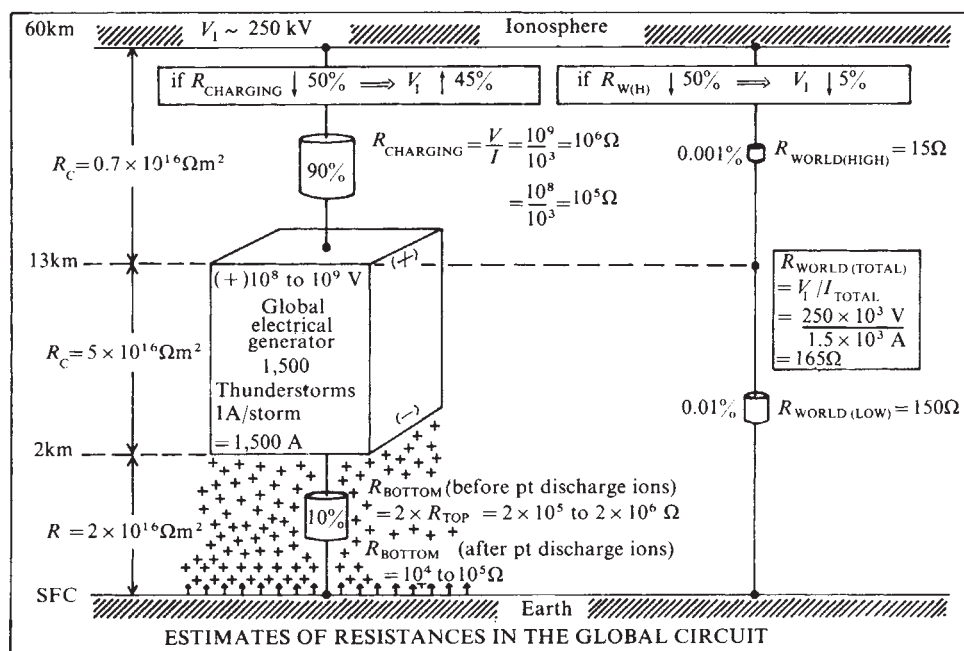
increase in conductivity occurs. This makes the resistance under the thunderstorm generator 10^4 – 10^5 Ω . Therefore, the resistance over the thunderstorm generator contains 90% of the total circuit resistance and the one under it contains 10%. The top load resistance is 0.001% and the bottom load resistance is 0.01% of the total circuit resistance. If the increase in conductivity under the thunderstorm generator only halved its columnar resistance, however, there would still be a large percentage (50%) of the total circuit resistance in the charging resistor. This is critical because if an appreciable portion of the total global circuit resistance is accessible to solar controlled ionising radiation, solar modulation of the Earth's electric field can be explained without changing the thunderstorm generator itself.

It may seem strange that the resistance over most of the Earth's surface is much lower than that of the air mass over thunderstorms; essentially this is a function of area. The atmosphere can be considered as an array of vertical columnar resistances. In fair-weather regions, all of these resistances in parallel result in a small total resistance; dividing a columnar resistance by a large cross-sectional area gives a small resistance. Over thunderstorms, the charging current flows through a resistor of relatively small cross-sectional area and the resistance is large.

The effect of ionising radiation variations on the charging resistor compared to the top load resistor is shown in Fig. 3. Initially it is assumed that the resistance over the thunderstorm generator decreases by 50%. As 90% of the total circuit resistance lies in this region, it would increase charging current by 45%. Therefore, if the load resistance remained constant, the ionospheric potential would increase by 45%. Alternatively, holding the charging resistance constant, a 50% decrease in the top segment of the load resistance would reduce it by 8 Ω , only 5% of the total load resistance. As most of the global circuit resistance is in the charging segment, a constant current generator may be assumed, and the IR drop across the return path would be 5% less than before. This would cause a 5% decrease in ionospheric potential. But, the assumed influx of ionising radiation would affect both the charging resistor and the top load resistor. Thus, summation of the two effects would result in a 40% (+45% – 5%) increase in ionospheric potential and fair-weather electric field intensity.

The possible influence of solar activity on currents flowing between thunderstorms and the ionosphere was mentioned by Fischer & Mühleisen⁵², and Hughes (personal communication).

Fig. 3 Details of the global circuit indicating how element resistances were derived and the distribution of resistance through the circuit. (Columnar resistances are not resistances relative to the thunderstorm generator *per se*; they must be divided by the applicable horizontal cross-sectional area to derive the resistance from top to bottom of an air column, see text).



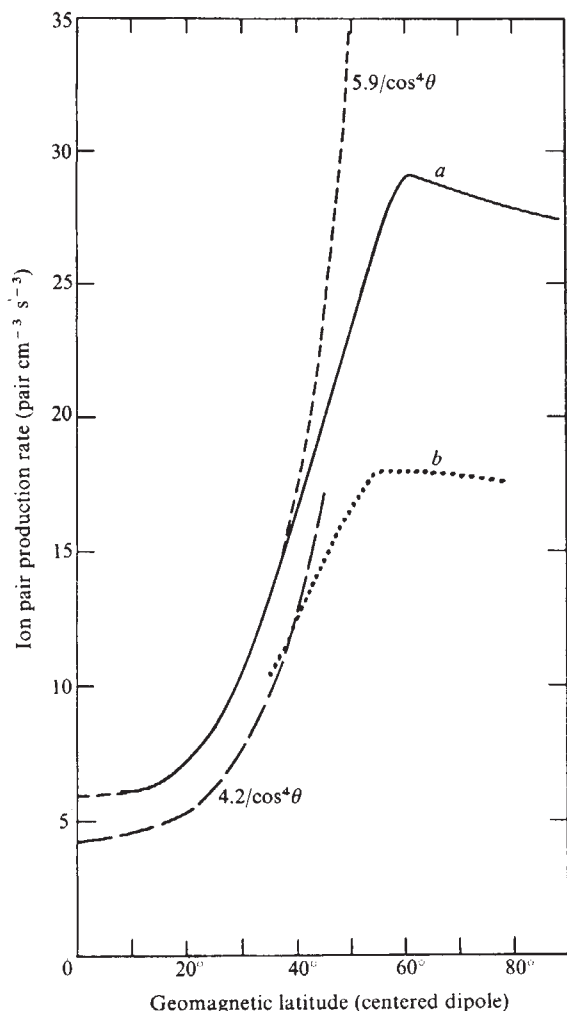


Fig. 4 Variation of ion production rate at 20 km as a function of geomagnetic latitude and solar activity. *a*, Solar minimum (1965); *b*, solar maximum (1958).

Proposed mechanism

Solar modulated variations in ionising radiation occurring in the volume above the thunderstorm generator change its resistance. With most of the global circuit resistance in this element, it is in effect a valve regulating the current flow in the entire circuit. As most variations in ionisation occur above 15 km, the charging resistor is affected, but there is little change in the total load resistance (only the top portion is accessible). The ionospheric potential and fair-weather electric field over most of the world must adjust in proportion to the variation in charging current. Thus, an increase in stratospheric ionisation over thunderstorms would cause a worldwide increase in electric field intensity which would explain reports of enhanced electric fields following solar flares. To explain an increase in thunderstorm activity, greater stratospheric ionisation and/or electric field intensity would need to amplify cloud electrification. Therefore, two possibilities for increasing thunderstorm electrification are: a direct local effect caused by increased ionising radiation over large cumulonimbus clouds; and an indirect worldwide effect due to increased electric field intensity in and near developing cumulus clouds.

The effects of atmospheric electricity on thunderstorm development or cloud physical processes are unknown. It has been suggested that initial electrical field intensity near developing cumulus controls their eventual electrification⁵³, while other mechanisms depend on transport of charge through the conductive region over the clouds^{54,55}. Increased electric field intensities within clouds could produce greater precipitation^{56,57}. Besides being an important effect (rainfall in many parts of the

world is almost entirely due to thundershowers) modulation of rainfall could affect cloud dynamics and atmospheric energetics.

Ionising radiation

It is important to consider the influence of latitude on access of particles to the atmosphere. As geomagnetic latitudes are displaced southwards of geographic latitudes by 12° maximum at the longitudes of Central and North America, this allows easier access of charged particles. Thus Caribbean thunderstorms at 20° geographic are at 32° geomagnetic and US thunderstorms at 40° geographic are at 52° geomagnetic. Solar corpuscular radiation (mostly protons) from solar flares takes between ~ 10 min and a few hours to reach Earth. These particles generally cannot penetrate the atmosphere below 10 or 15 km at high and mid-geomagnetic latitudes. Less energetic than galactic cosmic radiation, they are mostly deflected by the magnetosphere from the atmosphere at low magnetic latitudes. Galactic cosmic radiation reaches all latitudes. This flux of ionising radiation is modulated by variations of solar wind plasma in the interplanetary magnetic field. Therefore, solar activity can indirectly influence atmospheric ionisation over all the Earth's surface by regulating access of galactic cosmic radiation. Over a short term this is seen as 'Forbush decreases' following solar flares; over a long term there is an inverse correlation between the 11-yr solar cycle and atmospheric ionisation⁵⁸.

The variation in ion production rate at 20 km as a function of geomagnetic latitude and solar activity is shown in Fig. 4 (ref. 58). As expected, solar minimum is significantly larger than solar maximum. The variation exists even at low latitudes where the difference is about 35%. Because conductivity is proportional to the square root of ion production rate, it would vary by 10% on the average above the tops of equatorial thunderstorms through a solar cycle; at 60° geomagnetic (48° geographic over North America) the variation would be 70% for an increase in conductivity of 30% from solar maximum to solar minimum. If solar protons are screened at low latitudes, a direct solar influence on tropical thunderstorm electrification could be by modulation of galactic cosmic radiation leading to an out-of-phase correlation between the sunspot cycle and tropical thunderstorms. At high and mid-latitudes, where solar corpuscular radiation and particles from the aurora and magnetosphere have access to the lower stratosphere, an in-phase relationship would be expected. These phase relationships are suggested by Brooks' finding⁵⁹ (discussed in ref. 7) of high positive correlation between the sunspot cycle and high latitude thunderstorm activity compared to low, or negative, correlations for equatorial thunderstorms.

As thunderstorms frequently overshoot the tropopause by several km (ref. 60), the tops of equatorial cumulonimbus can approach 20 km. Ionising radiation reaching 25 km would affect enough of the resistance over these clouds to modulate their currents. At high to mid-latitudes, where the tropopause is lower, thunderclouds are correspondingly lower with most tops in the 10–16 km range. However, ionising radiation has easier access to the low stratosphere in these regions and penetration to 20 km would be sufficient to increase the current from many of these storms.

Holzworth and Mozer⁶¹ have shown that solar controlled ionising radiation variations can have large effects on conductivity down to at least 15 km at mid-geomagnetic latitude. Measurements were taken from balloons 30 km over Canada in a region where summer thunderstorms frequently occur (ref. 62 and R. H. Holzworth, personal communication). Figure 5 shows the first simultaneous measurements at one location of solar ionising radiation modulating atmospheric electric fields. A large decrease in electric field intensity (*b*) was recorded at the balloon coincident with simultaneous satellite observations of a solar proton event (*a*). As ionising radiation was also recorded which depicted a simultaneous large increase in radiation intensity (*c*), the local electric field decrease was caused by a corresponding increase in conductivity. Extra-

polating the data to lower altitudes, it was found that ionisation during the peak of the event exceeded the normal galactic-cosmic ray background down to at least 15 km. At that time there was an increase of two orders of magnitude in conductivity at 30 km.

Hoffman and Hopper⁶³ reported similar electric field and ionising radiation variations (on separate flights) at 20 km over Australia, a mid-geomagnetic latitude location.

Another possible important source of solar controlled stratospheric ionisation is charged particle precipitation from the magnetosphere and aurora during magnetic storms⁶⁴⁻⁶⁶. These particles produce bremsstrahlung X rays which ionise the atmosphere, sometimes down to 12 km (ref. 68). While confined to mid to high-geomagnetic latitudes (approximately north of the US-Canadian border) they would be over a region of significant thunderstorm activity.

Thunderstorm electrification

Increased conductivity over thunderstorms will cause more charge to flow to the tops of thunderclouds. Whether this will enhance the storm's ability to separate charge or not depends on the electrification mechanism, but there are many theories on this point. If convection is important, as proposed by Grenet⁶⁴ and Vonnegut⁶⁵, the electrification process would be accelerated. If increased currents are dissipative⁶⁸, which agrees with models where the cloud is electrified from within by hydrometeor interactions⁶⁹⁻⁷¹, the generator would weaken.

The intensity of the ionospheric potential and fair-weather electric field may also be important in thunderstorm electrification. Several charging mechanisms depend on polarisation of cloud droplets in the fair-weather electric field during the initial stages of electrification^{72,73}. Sartor⁷⁴ found that the ultimate electric field intensities in developing cumulus are extremely sensitive to the initial electric field strength. For a given fair-weather electric field which results in a weakly electrified cloud ($1,000 \text{ V m}^{-1}$), if the initial field intensity is increased by 32% (as might occur following solar activity), that cloud would become electrified to thunderstorm intensity ($100,000 \text{ V m}^{-1}$). An initial increase of electric field by 10% would result in an order-of-magnitude larger ultimate field intensity. The convective electrification mechanism may often be initiated by fair-weather monopolar space charge for initial cloud electrification before charging through conduction of fast ions to the upper portion of the cloud occurs. Fair-weather space charge density is proportional to fair-weather electric field intensity. Thus, for both induction and convection cloud electrification mechanisms, an increase in ionospheric potential and associated fair-weather electric field intensity could lead to electrification of more developing cumulus clouds and thunderstorms.

Cloud physical effects

Amplified electric fields in clouds may increase precipitation. High electric fields enhance coalescence of small droplets into large drops, the most important factor in rain formation. Even relatively weak fields of $1,000 \text{ V m}^{-1}$, one or two orders-of-magnitude less than those in thunderstorms, can enhance coalescence⁷⁵, although the effect is much greater with thunderstorm strength fields. Sartor⁷⁶ calculates that the instantaneous growth rate of precipitation particles goes up approximately proportionately with increased electric field intensity. Several physical processes that explain why electric fields affect coalescence have been summarised by Mason⁷⁷. Once droplets change to drops they can grow rapidly by collisions with other drops and become large enough to have a better chance of reaching the ground before evaporating. This would leave latent heat of condensation, released when the droplets formed from water vapour, in the atmosphere where it could affect circulation. Vonnegut⁷⁸ and Latham^{79,80} have reviewed ways in which electrification might influence cloud physical processes including nucleation and acceleration of ice crystal growth; both processes release latent heat.

Lightning may be an important atmospheric electrical stimulus for rain. Intense periods of rain—rainushes—are frequently associated in time and space with lightning flashes. While some believe that lightning results from hydrometeor interactions in the rainshaft, others suggest intensely electrified cloud particles, charged in the vicinity of the lightning, cause rapid coalescence of raindrops forming the rainush⁸¹. The drag on air caused by hydrometeors falling through the cloud, as well as the redistribution of ice and water in and near clouds, should influence cloud dynamics. The buoyancy of growing cumulus turrets will be greater if water is removed from them as there will be less evaporative cooling.

If there is an increase in thunderstorm clouds resulting from electrification of more developing cumulus and/or greater convection, another potentially important process may occur. Ice crystals from (1) the characteristic cirrus anvil which tops thunderstorms; and (2) middle altitude remnants of dissipated thunderclouds, carried downwind by the upper air motion, can seed nearby lower cloud layers. Middle and low clouds are generally present in the warm sector when frontal type thunderstorms occur. This process would stimulate rainfall resulting in the release of latent heat. Ligda⁸² has described how ice crystals from deep convective cloud remnants have apparently maintained steady rain for many hours from lower stratiform cloud layers. The extent to which electrical forces are important in cloud physical processes is unknown. It may have no effect or heavy rainfall may not occur without it⁷⁴.

Atmospheric circulation

How does solar control of thunderstorm electrification and resultant cloud physical effects, if these do occur, account for the widely reported response of Northern Hemisphere vorticity to solar sector crossings? Solar sectors were introduced

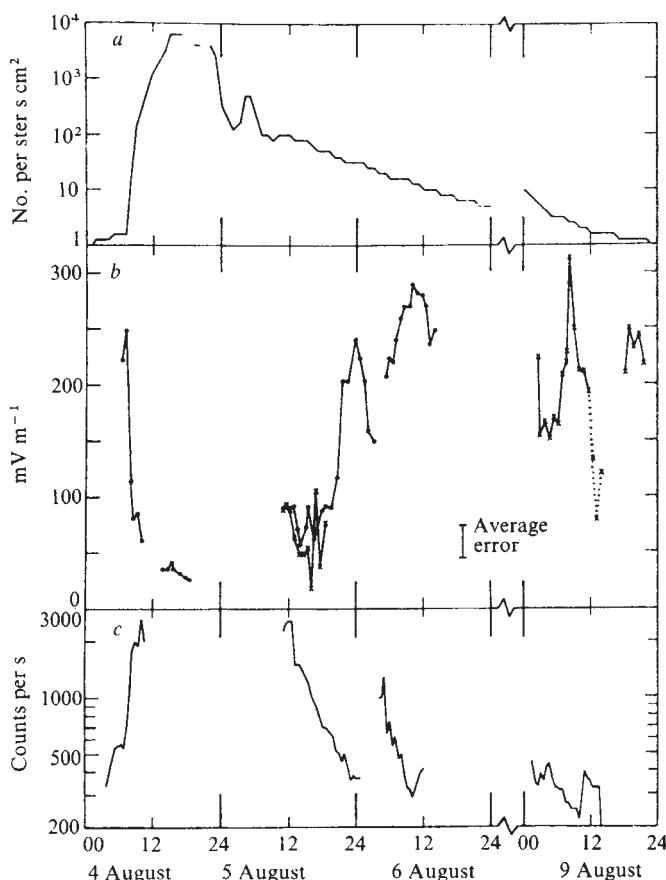


Fig. 5 Comparison of simultaneous measurements of solar protons from a satellite $E_p > 60 \text{ MeV}$ (a); vertical electric field from two balloons at 30 km over Canada, ●, Thompson; ×, College (b); and radiation intensity as recorded by X-ray detectors on the balloons (c). Solar flare occurred at 0617 on 4 August 1972.

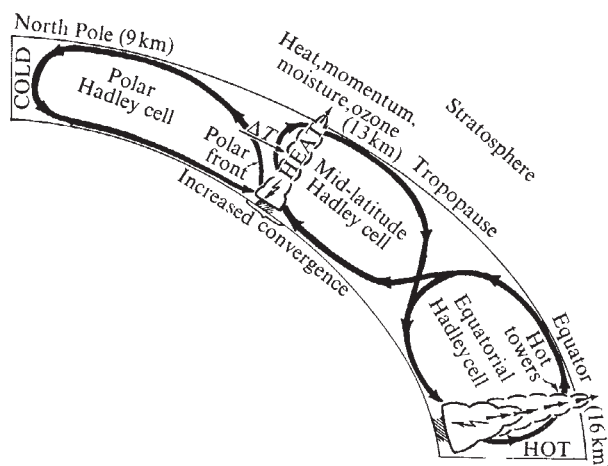


Fig. 6 Longitudinal cross-section of atmosphere illustrating how solar modulation of cloud physical processes might influence the general circulation.

as the solar controlled timing mark for use in Sun-weather studies, because both galactic and solar ionising radiation varied as a function of the Earth's position within these sectors⁷. The basic premise has been that from the lower stratosphere (25 km) down, ionisation is the only atmospheric parameter that changes appreciably as a function of solar activity; therefore an atmospheric electrical process should be the most likely candidate to explain a solar-weather effect.

Solar control of ionisation above thunderstorms may, however, ultimately modulate atmospheric energetics. Figure 6 shows how the general circulation might be influenced by atmospheric electrical modulation of cloud physical processes. Byers⁸³ states that the amount of rain is a direct measure of net release of latent heat to the atmosphere and that most tropical rain comes from thunderstorms. The equatorial Hadley cell, driven by a few 'hot towers' and/or the general upward motion in the intertropical convergence zone (ITCZ) would be energised if greater electrification of clouds in this belt led to more rainfall. This would speed up the meridional circulation as the equatorial cell helps drive the mid-latitude one. This would produce greater low-level convergence in the polar front region intensifying storminess and vorticity. Convergence would also be increased in the ITCZ so the process would have positive feedback. Because of the time it would take for the effect to propagate in the wind field, I estimate a delay of two or three days before increased low latitude convection would affect mid-latitude convergence. However, a pressure fluctuation, initiated by enhanced tropical convection, might propagate rapidly as a wave phenomenon to higher latitudes.

Increased meridional circulation implies blocking of the westerly zonal flow and more vorticity. Schuurmans⁸⁴ found meridional circulations are most frequent during highest solar activity, and Rasool⁸⁵ reported that the equatorial tropopause rises with increased solar activity. Enhanced deep convection in the ITCZ would raise the equatorial tropopause. Another possible atmospheric electrically-induced cause of vorticity intensification (with a time delay of a day or less) would be an increase in the north-south temperature gradient across the polar front. This could follow from more precipitation, caused (1) directly by electric forces; (2) indirectly by increased low-level convergence; or (3) by ice crystals from thunderstorms seeding lower clouds, in the pre-cold-front squall line region south-east of the front. In addition, intensification of thunderstorms would affect the vorticity pattern because they form a 'pressure dome' in the squall line region⁸⁶.

The idea that thunderstorms might influence vorticity may seem questionable as the winter (November-March) is when vorticity is most sensitive to solar sector crossings^{8,9} and we do not immediately think of mid-latitude thunderstorms as a winter phenomenon. While air-mass cumulonimbus are not common over continents during disturbed winter

weather, this does not mean winter storm clouds are not electrified. Snow clouds are charged⁸⁷, some sufficiently to produce lightning^{86,88}. Continental winter rainclouds are also charged⁸⁶. Electric fields may play an important part in winter storm precipitation. However, the key to explaining solar modulation of vorticity may be that oceanic thunderstorms maximise at northern latitudes in the winter^{88,89}. Convection over the ocean would be expected when deep layers of cold-continental air move over relatively warm water such as would be carried to the North Pacific by the Japanese Current and the North Atlantic by the Gulf Stream. These regions may provide the largest source of convective clouds accessible to solar controlled variations in ionising radiation (solar protons and X rays produced by charged particle precipitation). With (perhaps simultaneous) release of heat into the atmosphere over the North Pacific and North Atlantic, it is possible that the planetary Rossby wave, which circles the Earth at mid-latitudes and is the basic feature of the atmospheric circulation pattern, would be perturbed. Another geographical location with relatively easy access of ionising radiation to the low atmosphere is the 'South Atlantic magnetic anomaly'⁹⁰. In this region the Earth's magnetic field is weakest, and trapped particles in the magnetospheric radiation belts can come deepest into the atmosphere. Enhanced lightning frequency in this region has been reported^{91,92}. Ney⁹³ was one of the first to recognise that solar controlled variations in atmospheric ionisation might influence thunderstorm activity. But Sparrow and Ney⁹² found no difference between lightning frequency during solar maximum and minimum, however, the accuracy of their comparison was not better than a factor of two, more than the expected signal. They also found no relationship between lightning storm occurrence and the variation of the geomagnetic index K_p from one day before the storms through one day after them. Their satellite data extended only north and south of the equator by 35°.

Weather modification

If atmospheric electrical variations can influence weather, this suggests another way that man might tamper with nature. Atomic radiation released into the atmosphere by nuclear explosions changes conductivity in the stratosphere⁹⁴, and troposphere¹⁰³ as well as near the Earth's surface⁹⁵. High altitude nuclear explosions also inject charged particles into the magnetosphere where they are trapped in the Van Allen radiation belts increasing the source which can be precipitated by solar activity. The 'Starfish' event of 9 July 1962, and a similar Russian test in the same year, introduced such a high flux of energetic electrons into the magnetosphere that the normal radiation background was swamped⁹⁶; conditions did not return to normal until after 1966. Magnetospheric electrons constantly drizzle down into the stratosphere, but during times of geomagnetic activity they are injected in large quantities⁹⁷.

Control of charged particle precipitation by man is another possibility. It is known that VLF radio waves, such as those generated by lightning, can cause trapped particles to be dumped into the atmosphere by destabilising plasma in the magnetosphere⁹⁸. Experiments to cause such triggered particle precipitation have been conducted and others are planned from the space shuttle (T. F. Bell, personal communication).

Man-made changes in tropospheric conductivity should also be considered. Boeck⁹⁹ points out that if krypton 85, a radioactive gas from nuclear powerplants, continues to be released as planned, the global circuit will be altered within 50 years.

Conclusion

We have expanded the concept that a solar influence on weather might be through variation of conductivity in the lower stratosphere over thunderstorms¹⁰⁰, and described this mechanism within the framework of the atmospheric electrical global circuit.

If stratospheric ionisation modulates thunderstorm currents,

it follows that there should be a secular inverse correlation between the smoothed average ionospheric potential and the 11-yr sunspot cycle in accordance with the variation of galactic-cosmic radiation. Such a relationship is suggested by the balloon measurements of Mühleisen¹⁰¹ (see also ref. 100). Olson¹⁰² has reported the same inverse relationship from a decade of air-Earth current measurements from balloons.

On the other hand, a positive correlation between ionospheric potential and solar flares would be expected for short periods (days) following solar flares¹⁸⁻²⁰.

The argument that solar modulation of atmospheric conductivity would change the Earth's electric field intensity seems to be on firm ground both theoretically and from the results of

statistical studies. But the possibility of variable solar activity modulating thunderstorm activity, which in turn influences other parts of meteorology, is speculative and awaits further investigation.

I thank E. A. Bering for calling my attention to charged particle precipitation. R. Nagel pointed out the possible importance of thunderstorm ice particles seeding lower clouds. H. Dolezalek and B. Vonnegut provided valuable comments during revision. R. H. Holzworth and F. Mozer permitted reproduction of their data before publication. I also thank J. Hughes for support and encouragement. Support for this work was provided by the Office of Naval Research, Atmospheric Science Programme, under Contract N00014-74-C-0336.

Received 31 October 1977; accepted 3 March 1978.

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High sedimentation rates and variable dispersal patterns in the western Hellenic Trench

Daniel J. Stanley & R. John Knight

Division of Sedimentology, Smithsonian Institution, Washington, D.C., 20560

Robert Stuckenrath

Radiation Biology Laboratory, Smithsonian Institution, Washington, D.C., 20560

Gianpiero Catani

Istituto di Geologia, Università degli Studi, 34127-Trieste, Italy

The relationship between sedimentation and tectonics in island arc settings is a close one but remains poorly understood. A sedimentological investigation of the western Hellenic Trench has revealed some of the highest sedimentation rates measured in the Mediterranean Sea. The complex structural evolution and physiography of this region have given rise to a more complicated lithofacies pattern than that reported in most trench models.

THE western Hellenic Trench in the eastern Ionian Sea west of Greece is seismically one of the most active zones in the Mediterranean¹. The structural displacement affecting this sector of the Hellenic Trench during the Neogene and Quaternary produced a complex bottom physiography comprising basin plains, canyons, hummocky ('cobblestone') topography, ridges and steep slopes (Fig. 1). Unlike the continuous and elongate Pacific trenches, the Hellenic Trench in this region is an arcuate depression broken into a series of relatively small, disconnected basins that trend roughly parallel to the Hellenic Arc axis². The influence of geologically recent tectonics on the sediments in the trench, as revealed from high-resolution sub-bottom surveys³⁻⁵, show deformation of unconsolidated Plio-Quaternary series and older consolidated units, local ponding, and syndepositional tilting, folding and faulting of the uppermost deposits. The blocky and broken physiography resulting from this tectonic overprint (1) precludes the long distance transport of sediment by extent mass gravity processes; (2) fosters the restricted extent of individual turbidity current and other mass gravity flow deposits; and (3) results in irregular patterns of distribution of finer-grained turbid flow and suspension related units⁶. In addition to structural effects, the trench sediments and their patterns of dispersal record fluctuations of sediment input related to large-scale, basin-wide climatic and eustatic oscillations⁷ and to changes in water mass patterns⁸ during the Pliocene and Quaternary.

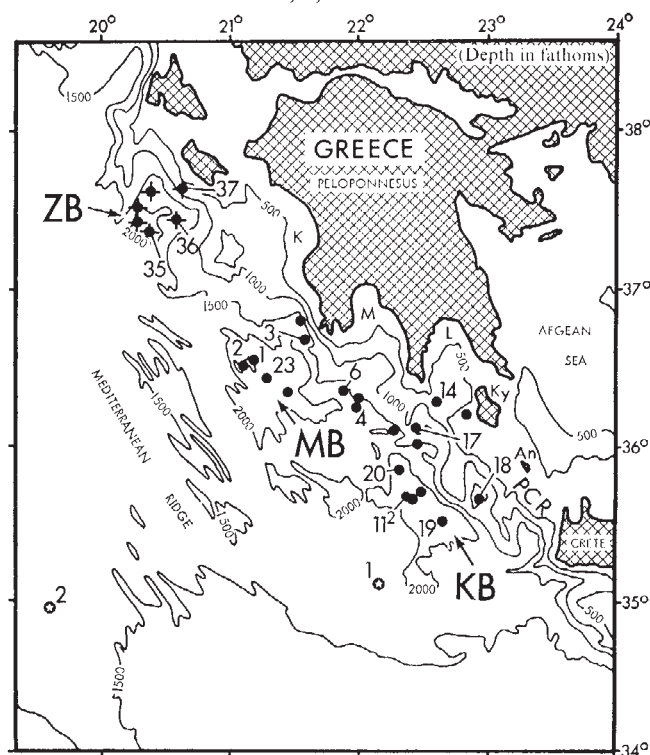
As a first step toward evaluating some of these effects in the late Quaternary, a stratigraphic study of 28 piston cores from the slope and basin environments of the Hellenic Trench was initiated west of the Peloponnese. Twenty-six cores were collected on three cruises (USNS Lynch-1972; RV Trident-1975; RV Marsili-1976) in the three major structural-depositional systems forming the western Trench (Fig. 1): Zakynthos-Strofoládes (ZB) basin-slope, 'Matapan Deep' (MB) basin-slope, and Kythera-Antikythera (KB) basin-slope complex. The three individual basin-slope systems are orientated roughly parallel to the major trend (NW-SE) of this sector of the Hellenic tectonic arc. The

'Matapan Deep' (MB) with a maximum depth slightly greater than 5,000m is the deepest part of the Mediterranean⁹; the other two basins (ZB and KB) are 4,148m and 4,614m deep respectively¹⁰. Two additional cores (Lynch II-1,2), collected on the Mediterranean Ridge west of the Trench, were examined for stratigraphic comparison. Thirty-six radio-carbon dates, obtained from 17 cores (Fig. 2), provides an absolute age framework for stratigraphic correlation.

Core logs and stratigraphy

The simplified core logs (Fig. 2) reveal that the lithology in the trench is extremely variable. It is difficult, if not impossible in some cases, to correlate cores from basin to basin, or even within the same sector of the trench on the basis of lithology alone. Furthermore, stratigraphic zonation in this region based on isotopic and microfossil markers is also somewhat arbitrary¹¹. Radiocarbon dates are necessary

Fig. 1 Western Hellenic Trench and adjacent area in Ionian Sea, eastern Mediterranean, showing locations of cores used in study. ◆, Trident cores (6); ●, Marsili cores (20); starred circles, Lynch-II cores (2). Ky, Kythera island; An, Antikythera island; PCR, Peloponnese-Crete Ridge; K, Gulf of Kiparissia; M, Gulf of Messenia; L, Gulf of Laconia.



to accurately identify stratigraphically important isochrons, such as dark, organic-rich sapropel layers and volcanic ash or tephra layers, that were deposited over large areas in the eastern Mediterranean. Recognition of these more prominent late Quaternary stratigraphic units permits more reliable correlation of cores within the trench and with other regions of the eastern Mediterranean.

The two Lynch cores on the Mediterranean Ridge, west of the Trench, display partially complete lithologic sequences (Fig. 2) that include the Ischia tephra (here identified petrographically on the basis of refractive index and dated by other workers¹¹ at about 25,000 yr BP) between two sapropels, interpreted as S_1 and S_3 . The upper three sapropels have been radiocarbon dated at about 7,000–9,000 yr BP (S_1), 23,000–25,000 yr BP (S_2) and 38,000–40,000 yr BP (S_3) in the Levantine Basin¹². Thus, the stratigraphic sequences in these two Ridge cores can be correlated with other cores collected outside the Trench in the Ionian and Levantine basins^{13–15}, and represent sediment that accumulated during the last major eustatic cycle (sealevel lowering from ~ 30,000 yr BP to ~ 17,000 yr BP, and then rising rapidly to ~ 6,000 yr BP (ref. 12)). As might be expected, a large proportion of the sediment that was deposited during the Quaternary on the Ridge, being some distance from land, was derived primarily from suspension-related transport mechanisms. The calculated sedimentation rates in the Lynch cores was about 7–9 cm kyr⁻¹ during the late Pleistocene, decreasing to about 2–3 cm kyr⁻¹ during the Holocene (Fig. 3, Ridge). Comparable rates were measured in the Quaternary sections of the deep-sea drilling project, Leg 13, cores 125 and 126 on the ridge, drilled close to the location of the two Lynch cores³.

Rates of Sedimentation

Broadly similar stratigraphic sequences were observed in the cores from the western Hellenic Trench (Fig. 3, ZB, MB and KB). Here the high sedimentation rates, recorded during the period before 20,000 yr BP, are probably related to relatively high fluvial discharge and increased sediment transport across the then subaerially exposed shelf to the west of the Peloponnese. Lowered sealevel stands at times may have been coincident with more pluvial conditions. Following this period, fluvial discharge and offshore sediment transport decreased as sealevel rose during the early Holocene. The marked decrease in sedimentation rates with time, associated with regionally important climatic-eustatic fluctuations, is observed over most Mediterranean margins^{12,16–18}.

The considerable contrast between the rather constant rates of sedimentation noted on the Ridge (Fig. 3) and the high core-to-core variability within the Trench is significant. The large differences within individual trench basins reflect lateral differences of input, accessibility, and transport processes. Depositional patterns on the Ridge more closely reflect uniform accumulation from suspension-related, hemipelagic processes, while those of the physiographically complex trench system, to a large degree, record the effects of physiographic barriers, that is sediment dispersal patterns from both gravity and suspension mechanisms are modified by ridges, slope basins and 'cobblestone' topography⁶. In spite of these barriers, sedimentation rates in the basin plains of the Trench are high (Fig. 3, see cores T35, T36, M1, M20, M23) which suggests that at least some parts of the three major trench basins serve as ultimate catchments of both hemipelagic and gravity-assisted deposits (Knight and Stanley, in preparation). The highly variable rates measured within the same basin-slope system (compare the rates of M20 with those of M11-2 and M19 in the Kythera-Antikythera basin, Fig. 3) seem to be anomalous. The apparent inconsistencies can be explained from an examination of the cross-sectional configuration of the Trench on 3.5 kHz and seismic profiles. These records show that the

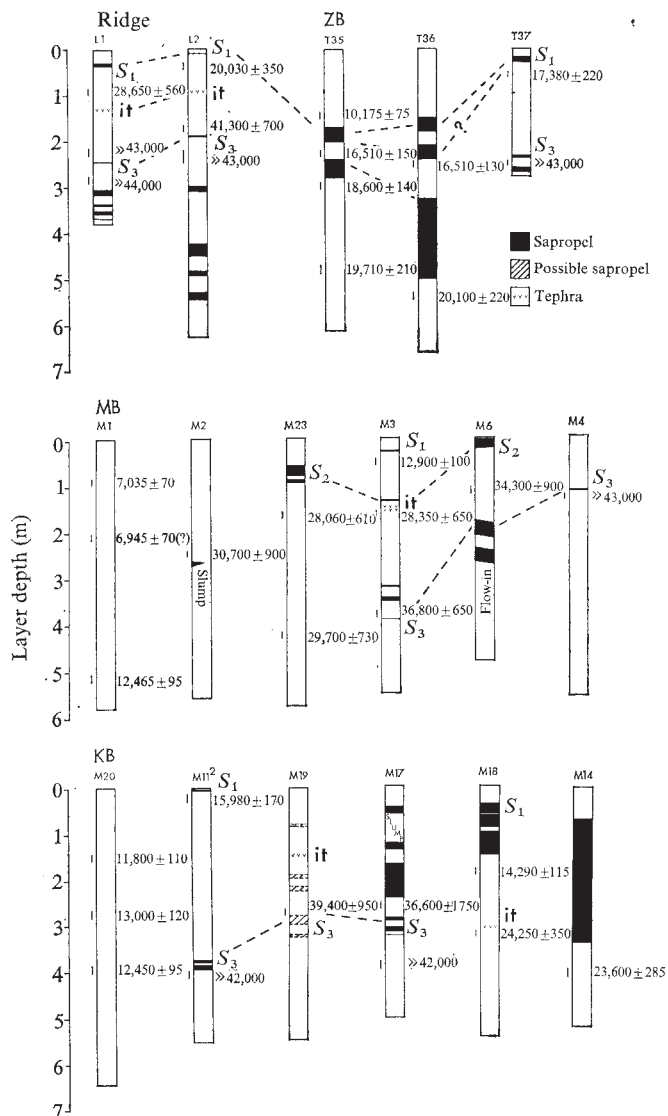


Fig. 2 Simplified core logs showing sapropel (code S_1 , S_2 , S_3 after ref. 12) and tephra layers. S_1 , upper sapropel (~ 7,000–9,000 yr BP); S_2 , second sapropel (23,000–25,000 yr BP); S_3 , third sapropel (38,000–> 40,000 yr BP); and it, Ischia tephra (about 25,000 yr BP after ref. 11).

western Hellenic Trench does not conform to the classic 'V' shape in transverse section as commonly envisaged for trenches, but is broken by an irregular series of structural highs and lows⁹.

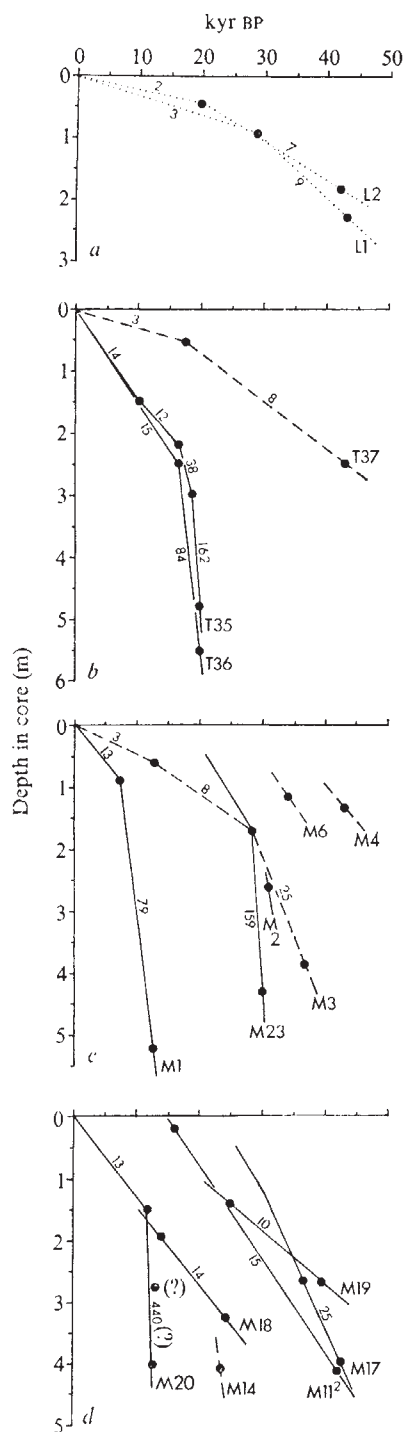
Cores from the slopes generally record lower sedimentation rates (Fig. 3). This is probably a function of truncated or missing sections (for example, M3, M4, M6, T37) that are the result of (1) failure by slumping (M17), (2) non-deposition and/or non-accumulation on steep slopes due to sediment by-passing directly to more distal, less steep environments, or (3) inaccessibility due to physiographic barriers (that is damming and diversion by ridges and other bathymetrically positive areas upslope). Cores retrieved from slope basins (for example, cores M17 and M18) or in major embayments, such as the large valley off the Gulf of Laconia (core M14), tend to show somewhat higher rates and more complete stratigraphic sections.

Lithological variations

The calculated sedimentation rates help to explain some of the observed lithological variations from the trench sediments. Sapropels from trench basins, for example, tend to be thicker, somewhat less distinct (dark olive-grey) and more broken by hemipelagic sediments in the Hellenic Trench

than those on the Mediterranean Ridge (thin, black and unbroken). Thicker sapropels occur in some cores showing moderate to high ($>25 \text{ cm kyr}^{-1}$) sedimentation rates (for example, T36, M14) but are absent in areas with the highest calculated sedimentation rates (for example, M1, M20). The lithological anomalies may be the result of dilution by high sediment input from frequent and/or thick gravity flows that mask the dark layers. This phenomenon might also explain why some sapropels are broken into several layers and/or units (for example, the upper sapropel in core T36,

Fig. 3 Computed sedimentation rates (in cm kyr^{-1}) based on radiocarbon dates of core samples shown in Fig. 2. *a*, Ridge, Mediterranean Ridge; *b*, ZB, Zakynthos-Strofádhes sector; *c*, MB, Matapan sector; and *d*, KB, Kythera-Antikythera sector. L2, Core no.; ●, dated sample; solid line, trench-basin system; dashed line, slope; dotted line, ridge; number over sloping line indicates rate of sedimentation (cm kyr^{-1}).



above the sediment sample dated at $16,510 \pm 150 \text{ yr BP}$ is probably S_1 , split into two units).

In some instances, a sapropel-like layer occurs at an unexpected stratigraphic level. The lower sapropel in core T35 is bounded by samples dated at 16,510 and 18,600 yr BP which is too old to be S_1 (7,000–9,000 yr BP) and too young to be S_2 (dated at $\sim 23,000$ – $25,000 \text{ yr BP}$ (ref. 12)). This type of sapropel occurrence may indicate the influence of local stratification and restriction of water mass flow producing euxinic conditions within deep trench basins. The presence of stratigraphically anomalous sapropels may also suggest downslope emplacement of Quaternary sections from slumping (for example, cores M2 and M17). Such mass failure effects might be expected to occur on relatively steep slopes with high sedimentation rates in seismically active regions.

The overall regional differences in the rates of sedimentation from the north-west to the south-east along the Trench should also be noted. The Zakynthos-Strofádhes and Matapan sectors seem to have received a greater supply of terrigenous sediments, at least during the late Quaternary, than the Kythera-Antikythera zone. Sediments derived from the western and southern Peloponnese from rivers, such as the Alpheios, Evrotas and Kalamata, were moved seawards through large canyons extending from the Gulfs of Kiparissia, Messinia and Laconia (Fig. 1, K, M and L) and deposited directly on slopes and/or the trench basins. The Peloponnese-related provenance pattern is in marked contrast to that of the Kythera-Antikythera sector which is bounded to the northeast by the Peloponnese-Crete Ridge (Fig. 1, PCR). This ridge, with the islands of Kythera (Ky) and Antikythera (An), serves as a dam between the Aegean and Ionian Seas. A sub-bottom survey in the southern Aegean shows the presence of up to 1,000m of Plio-Quaternary sediments ponded on the north-east side of the ridge (PCR)¹⁹.

Conclusions

Cores from the western Hellenic Trench show some of the highest sedimentation rates yet measured in the Mediterranean Sea. The late Quaternary depositional pattern is one of limited source areas with fluvial systems providing moderate volumes of sediment to a series of relatively small, topographically isolated catchment sites that have been subjected to almost continuous syndepositional tectonic displacement and deformation. The resulting complex bottom physiography of the western Hellenic Trench has given rise to a highly variable pattern of sediment dispersal and considerably more complicated lithofacies patterns than those reported in some trench models^{20,21}. A dense coring programme, with stratigraphic analysis based on absolute age determinations, is essential for interpreting the sedimentation history of the Hellenic Trench.

We thank the US Naval Oceanographic Office, the Italian CNR, and the universities of Trieste, Rhode Island and Perpignan for assistance in the collection of the cores used in this study. We thank A. Brambati and R. Marocco (Trieste), H. Got (Perpignan) and T. C. Huang (Rhode Island) for help in planning and conducting the work at sea. H. Sheng assisted in the identification of tephra layers. This study, part of the Mediterranean Basin (MEDIBA) Project, was funded by Smithsonian Research Foundation grant 71702120.

Received 17 January; accepted 21 February 1978.

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Complete nucleotide sequence of SV40 DNA

W. Fiers, R. Contreras, G. Haegeman, R. Rogiers, A. Van de Voorde,
H. Van Heuverswyn, J. Van Herreweghe, G. Volckaert & M. Ysebaert

Laboratory of Molecular Biology, University of Ghent, Belgium

*The determination of the total 5,224 base-pair DNA sequence of the virus SV40 has enabled us to locate precisely the known genes on the genome. At least 15.2% of the genome is presumably not translated into polypeptides. Particular points of interest revealed by the complete sequence are the initiation of the early *t* and *T* antigens at the same position and the fact that the *T* antigen is coded by two non-contiguous regions of the genome; the *T* antigen mRNA is spliced in the coding region. In the late region the gene for the major protein VP₁ overlaps those for proteins VP₂ and VP₃ over 122 nucleotides but is read in a different frame. The almost complete amino acid sequences of the two early proteins as well as those of the late proteins have been deduced from the nucleotide sequence. The mRNAs for the latter three proteins are presumably spliced out of a common primary RNA transcript. The use of degenerate codons is decidedly non-random, but is similar for the early and late regions. Codons of the type NUC, NCG and CGN are absent or very rare.*

SIMIAN virus 40 (SV40) (and the distantly related polyoma virus) have been intensively studied both as models of eukaryote gene organisation and expression and because of their oncogenic potential (see refs 1–3 for recent reviews). SV40 infection in monkey cells follows a lytic cycle but in other cells such as mouse, rat, rabbit and human the infection is abortive and the SV40 genome or parts thereof can become integrated into the host cell genome resulting in transformation of the cell. SV40 can also cause tumours when inoculated into newborn animals. The determination of the complete sequence reported here now makes it possible to assign precise genomic locations to biological functions that have been discovered and analysed by many workers and which will be discussed below.

The SV40 virion contains, in addition to its genome wrapped in the host-derived histones H2A, H2B, H3 and H4, the major capsid protein VP₁ and two minor structural components, VP₂ and VP₃. SV40 DNA is a supercoiled, circular duplex of 5,224 base pairs. The single *EcoRI* restriction enzyme site is taken as zero point for the physical map (Fig. 1). The restriction enzymes *HindII* and *III* produce 13 fragments designated A to M^{1,5}, which are often used as a basis for further studies. The cleavage sites of many

other restriction enzymes have been determined^{1,2,6}. DNA replication initiates bidirectionally from around position 0.67 and terminates on the other side of the circle, where the two replication forks meet. The half-circle from 0.67 to 0.17, replicated and transcribed counterclockwise, corresponds almost exactly to the early region, while the segment 0.67 to 0.17 replicated and transcribed clockwise, corresponds to the late region (Fig. 1).

We report here the entire nucleotide sequence of SV40 DNA (strain 776). The earlier results were obtained by analysing highly labelled RNA transcribed *in vitro* from restriction fragments^{7,8}. The introduction by Maxam and Gilbert⁹ of the base-specific, partial chemical degradation of terminally labelled DNA, however, allowed much faster sequence analysis. All our more recent results were obtained by this approach, often applied on each of the two complementary strands. S. M. Weissman and his colleagues have pursued similar lines of investigation and their results are presented elsewhere (ref. 10 and references therein).

For representation of the sequence we have taken as a zero point the base pair present in the centre of the remarkable, 27-base-pair long palindrome located at position 0.663 (ref. 11). This centre lies at or very close to the origin of DNA replication. The single site on SV40 DNA for the restriction enzyme *BglI* is also around this position (the enzyme recognises the central part of the palindrome mentioned above). The nucleotide sequence of the early region is presented in Fig. 2 and the sequence of the late region in Fig. 3. For both regions, only the DNA strand with the same polarity as the messenger is given. This corresponds to a counterclockwise and a clockwise orientation respectively (see Fig. 1). The endpoint at the junction of the *Hind* fragments C and B is somewhat arbitrary; it should be noted, however, that the end of the cytoplasmic late mRNA corresponds almost exactly to this position (see below).

The early region

Soon after infection of either permissive or non-permissive cells by SV40, virus-specific, poly(A)-containing 19S mRNA appears in the cytoplasm¹². This early mRNA is transcribed counterclockwise and hybridises to about 48% of the genome from approximately 0.65 to 0.17 (refs 13, 14). The same region is also expressed in SV40-transformed cells, although transcription may proceed somewhat further in the 3'-end direction¹⁵. Temperature-sensitive mutants affected in an early function correspond to the complementation group A, and these mutations map in the *Hind* fragments H, I, and occasionally B^{16,17}. Gene A codes for

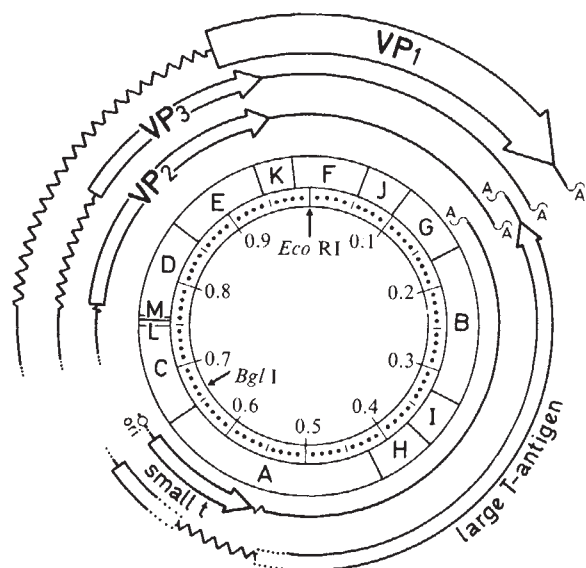


Fig. 1 Standard physical map of SV40 DNA and localisation of the main biological functions. The single cleavage site of the restriction enzyme *EcoRI* is used as a reference point for the physical map (inner circle). The next circle shows the position of the *HindIII* and *III* restriction fragments A to M. These *HindIII* cleavage sites (and *EcoRI*) are also indicated on the total nucleotide sequence in Figs 2 and 3. The origin of DNA replication (*ori*) is at or close to 0.663; this site corresponds to a large palindromic sequence, part of which represents the recognition sequence of *BglII*. The centre of this palindrome is taken as the zero point for presenting the sequence of the early region (5'-3' in counterclockwise orientation) in Fig. 2 and of the late region (5'-3' in clockwise orientation) in Fig. 3. The five virus-coded proteins are indicated by blocked arrows; note that the T antigen is coded by two non-contiguous segments on the genome (there is some uncertainty in the position of the points as indicated by the dashes). Untranslated parts of the mRNA are shown as solid lines; dots indicate uncertainty as to the exact position of the 5' end. Zigzag lines are used for the segments spliced out. A wavy line with an A illustrates the 3'-terminal polyA tail.

the T antigen, a protein with a molecular weight of about 90,000–100,000 (ref. 18). It is phosphorylated but presumably not glycosylated (at least not the nuclear T antigen¹⁹). T antigen is found in productive infections and in SV40-transformed cells. In the former, it is amongst other things essential for SV40 replication and for stimulating cellular functions; in non-permissive cells, T antigen is required for the initiation and presumably maintenance of transformation^{1–3}. T antigen is an autoregulator; it shuts off its own synthesis at the level of transcription^{20,21}.

We have localised the carboxyterminal end of the T antigen at nucleotide 2,549 of the early region (Fig. 2 and ref. 22). The exact position of the N-terminal starting signal of this gene, however, has only recently become clear^{23,24}. Indeed, originally there was some confusion because the region between map positions 0.54 and 0.175 can code for, at most, a polypeptide of molecular weight 72,000 while the estimated size of T antigen exceeds 90,000. The reasons for placing the boundary at about map position 0.54—and not closer to the origin—were based on the isolation and characterisation of a series of non-defective deletion mutants which lack sequence information in the region 0.54–0.59 and which still produce T antigen of normal size^{25,26}. Moreover, nucleotide sequence information in the region immediately preceding 0.54 clearly indicated that translation termination codons are present in all three possible reading frames, thereby precluding continuous protein synthesis through this region^{6,27}.

Progress in the characterisation of polyoma virus (which infects mice) has revealed that its molecular biology is in many aspects similar to that of SV40^{1,2}. T. Benjamin and

coworkers have isolated and characterised what seems to be a new class of early mutants^{28,29}. These are host range mutants growing normally only on certain cell types; they can no longer transform cells. They have been mapped in the proximal part of the early region³⁰, in a segment which may approximately correspond to the 0.54–0.59 area of SV40 DNA. Indeed, the SV40 mutants mentioned above which have a deletion in this region cannot transform cells to anchorage-independent growth³¹. The protein responsible for this second early function was subsequently identified and called small t antigen^{26,32,33}. It has a molecular weight of 15,000–20,000 and is absent or truncated in nearly all deletion mutants which map between 0.54–0.59 (refs 26, 34, 35). A remarkable fact is that T and t are related; they share immunogenic determinants and most of the methionine-containing tryptic peptides of t are also present in the tryptic fingerprint of T. Both proteins normally have a blocked N-terminus, which impedes further detailed analysis. Paucha *et al.*²³, however, were able to synthesise unblocked polypeptides *in vitro*. t and T were found to have an identical N-terminal sequence. The start codon for both proteins was identified by correlating the protein chemistry data with the nucleic acid sequence^{23,24}. t Starts at nucleotide 80 of the early region (Fig. 2) and is read continuously until the stop codon UAA at position 602. We assume a colinearity between the gene and the protein which is likely since Paucha *et al.*²³ were able to synthesise apparently normal t (but not T) using complementary RNA transcribed *in vitro* from SV40 DNA with *Escherichia coli* RNA polymerase.

t Is 174 amino acids long and has a molecular weight of 20,503; the sequence is shown in Fig. 4. The N-terminal methionine is blocked by acetylation (A. Mellor and A. E. Smith, personal communication). t Contains 19 lysine and 8 arginine residues and 14 aspartic acid and 11 glutamic acid residues; hence it is slightly basic. Most noteworthy is the high content of S-containing amino acids: 10 methionine and 11 cysteine residues. The latter are present mainly in the second half of the molecule. Except for its essential role in transformation (possibly involving acetylation of histones amongst other things³⁶), little is known as yet concerning the biological function or mechanism of action of t.

T starts at the same position on the genome as t^{23,24} and both molecules are identical up to perhaps about the middle of t. Then, due to a crossover, the remaining part of T is coded by a region starting around map position 0.53 (see below) and terminating with the UAA codon at position 2,550 (Fig. 2). Such a mechanism whereby the synthesis of a polypeptide is directed by two non-contiguous segments on the genome is, of course, formally equivalent to the insertion of extraneous DNA into a structural gene^{37,38}. The crossover in the t region cannot occur much before nucleotide 280 (Fig. 2) since otherwise the number of identical peptides in t and T could not be accounted for; on the other hand, the crossover cannot occur beyond nucleotide 380 since this region can be deleted in viable mutants which still make normal T but not t^{25,26,34,35}. T can be coded again from nucleotide 672 onward, that is, a continuous reading frame is present until the termination codon at position 2,550. The deduced amino acid sequence is shown in Fig. 4. It is not known at which point in the nucleotide sequence translation actually picks up again, but we believe it to be not too far from the earliest possible position mentioned above. Indeed, the mutant dl-1001 lacks the *HindIII* fragments H and I and induces the synthesis of a shortened, T antigen-related protein with a molecular weight of 33,000 (ref. 18). Since these two *HindIII* fragments code for 20,400 molecular weight of protein, it seems likely that the deleted DNA is not a multiple of three nucleotides and, as a result, translation after the deletion must continue in another reading frame and stop soon after the *HindIII* B

C G C C T C G G C C T C T G A G C T A T T C C A G A A G T A G T G A G G A G G C T T T T T G G A G G C C T A G G C T T T
 T G C A A A A A G C T T T G C A A A G A T G G A T A A A G T T T A A A C A G A G A G G A A T C T T T G C A G C T A A T
 G G A C C T T C T A G G T C T T G A A A G G A G T G C C T G G G G A A T A T T C C T C T G A T G A G A A A G G C A T A
 T T T A A A A A A A T G C A A G G A G T T T C A T C C T G A T A A A G G A G G A G A T G A A G A A A A A T G A A G A A
 A A T G A A T A C T C T G T A C A A A A A T G G A A G A T G G A G T A A A A T A T G C T C A T C A A C C T G A C T T
 T G G A G G C T T C T G G G A T G C A A C T G A G G T A T T T G C T C T T C C T T A A A T C C T G G T G T T G A T G C
 A A T G T A C T G C A A A C A A T G G C C T G A G T G T G C A A A G A A A A T G T C T G C T A A C T G C A T A T G C T T
 G C T G T G C T T A C T G A G G A T G A A G C A T G A A A A T A T A C A G G A A A G A T C C A C T T G T
 G T G G G T T G A T G C T A C T G C T T C G A T T G C T T T A G A A T G T G G T T T G G A C T T G A T C T T T G T G A
 A G G A A C C T T A C T T C T G T G G T G T G A C A T A A T T G G A C A A A C T A C C T A C A G A G A T T A A A G C T
 C T A A G G T A A A T A T A A A A T T T T A A G T G T A T A A T G T G T T A A A C T A C T G A T T C T A A T T G T T
 G T G T A T T T T A G A T T C C A A C C T A T G G A A C T G A T G A A T G G G A G C A G T G G T G G A A T G C C T T T A
 A T G A G G A A A A C C T G T T T T G C T C A G A A G A A A T G C C A T C T A G T G A T G A T G A G G C T A C T G C T G
 A C T C T C A A C A T T C T A C T C C C C A A A A A G A A G A G A A G G T A G A A G A C C C A A G G A C T T T C
 C T T C A G A A T T G C T A A G T T T T T G A G T C A T G C T G T T T A G T A A T A G A A C T C T T G C T G C T
 T T G C T A T T T A C A C C A C A A A G G A A A A A G C T G C A C T G C T A T A C A A G A A A A T T A T G G A A A A A
 A T T C T G T A A C C T T T A T A A G T A G G C A T A A C A G T T A T A A T C A T A A C A T A C T G T T T T T C T T A
 C T C C A C A C A G G C A T A G A G T G T C T G C T A T T A A T A A C T A T G C T C A A A A A T T G T G T A C C T T T A
 G C T T T T A A T T G T A A A G G G G T T A A T A A G G A A T A T T T G A T G T A A G T G C C T T G A C T A G A G
 A T C C A T T T T C T G T T A T T G A G G A A A G T T T G C C A G G T G G G T T A A A G G A G C A T G A T T T A A T C
 C A G A A G A A G C A G A G G A A A C T A A A C A A G T G T C C T G G A A G C T T G T A A C A G A G T A T G C A A T G G
 A A A C A A A A T G T G A T G A T G T G T T T A T T G C T T G G G A T G T A C T T G G A A T T T C A G T A C A G T T
 T T G A A A T G T G T T T A A A A T G T A T T A A A A A G A A C A G C C C A G C A C T A T A A G T A C A T G A A A
 A G C A T T A T G C A A A T G C T G C T A T A T T T G C T G A C A G C A A A A C C A A A A A C C A T A T G C C A A C
 A G G C T G T T G A T A C T G T T T A G C T A A A A A G C G G G T T G A T A G C C T A C A A T T A A C T A G A G A A C
 A A A T G T T A A C A A C A G A T T T A A T G A T C T T T T G G A T A G G A T G G A T A T A A T G T T T G G T C T A
 C A G G C T C T G C T G A C A T A G A A G A A T G G A T G G C T G G A G T T G C T T G G C T A C A C T G T T T G T T G C
 C C A A A A T G G A T T C A G T G G T G T A T G A C T T T T A A A A T G C A T G G T G T A C A A C A T T C C T A A A A
 A A A G A T A C T G G C T G T T A A G G A C C A A T T G A T A G T G G T A A A C T A C A T T A G C A G C T G C T T
 T G C T T G A A T T A T G T G G G G G A A A G C T T T A A A T G T T A A T T T G C C C T T G G A C A G G C T G A A C T
 T T G A G C T A G G A G T A G C T A T T G A C A G T T T T A G T A G T T T T G A G G A T G T A A A G G C A C T G
 G A G G G A G T C C A G A G A T T G C C T T C A G G T C A G G G A A T T A A T A C C T G G A C A A T T A A G G G
 A T T A T T T G G A T G G C A G T G T T A A G G T A A A C T T A G A A A G A A A C A C C T A A A T A A A A G A A C T C
 A A A T A T T T C C C C T G G A A T A G T C A C C A T G A A T G A G T A C A G T G T G C C T A A A A C A C T G C A G G
 C C A G A T T T G T A A A A C A A A T A G A T T T A G G C C C A A A G A T T A T T A A A G C A T T G C C T G G A A C
 G C A G T G A G T T T T T G T T A G A A A G A G A A T T C A A A G T G G C A T T G C T T T G C T T C T T A T G T
 T A A T T T G G T A C A G A C C T G T G C T G A G T T T G C T C A A A G T A T T C A G A G C A G A A T T G T G G A G T
 G G A A A G A G A G A T T G G A C A A A G A G T T T A G T T T G C A G T G T A T C A A A A A T G A A G T T T A A T G
 T G G C T A T G G A A T T G G A G T T T A G A T T G G C T A A G A A C A G T G A T G A T G A T G A A G A C A
 G C C A G G A A A T G C T G A T A A A A T G A A G A T G G T G G G G A G A A G A A C A T G G A A G A C T C A G G C
 A T G A A A C A G G C A T T G A T T C A C A G T C C C A A G G C T C A T T T C A G G C C C T C A G T C C C T C A C A G T
 C T G T T C A T G A T C A T A A T C A G C C A T A C C A C A T T T G T A G A G G T T T A C T T G C T T A A A A A C
 C T C C C A C A C C T C C C C T G A A C C T G A A A C A T A A A A T G A A T G C A A T T G T T G T T G T T ... 3'

Fig. 2 Nucleotide sequence of the early region. Only the strand with the same polarity as the early mRNA is shown (in a 5' to 3' orientation). The sequence starts at the centre of the 27 base pair long palindrome at position 0.663 on the standard map (*Bgl*I site). The end is the junction between the *Hind* fragments B and G. The different *Hind* fragments are indicated. The initiation codon for t and T antigens is boxed, as are the respective termination codons. The reading frame for translation is indicated by dots (see text, and Figs 1 and 5 for the special role of the sequence between about nucleotide 380 and 672).

G G C C T C G G C C T C T G C A T A A A T A A A A A A A T T A G T C A G C C A T G G G G C G G A G A A T G G G C G G A A
 C T G G G C G G A G T T A G G G G C G G G A T G G G C G G A G T T A G G G G C G G G A C T A T G G T T G C T G A C T A A
 T T G A G A T G C A T G C T T T G C A T A C T T C T G C C T G C T G G G G A G C C T G G T T G C T G A C T A A T T G A G
 A T G C A T G C T T T T G C A T A C T T C T G C C T G C T G G G G A G C C T G G G A C T T T C C A C C C C T A A C T G
 A C A C A C A T T C C A C A G C T G G T T C T T T C G C C T C A G A A G G T A C C T A A C C A A G T T C C T C T T T C
 A G A G G T T A T T T C A G G C C A T G G C T G C G C C G G C T G T C A C G C C A G G C C T C C G T T A A G G T T C G T
 A G G T C A T G G A C T G A A A G T A A A A A A C A G C T C A A C G C C T T T T G T G T T T G T T T A G A G C T T
 T T G C T G C A A T T T T G T G A A G G G G A A G A T A C T G T T G A C G G G A A A C G C A A A A A C C A G A A A G G
 T T A A C T G A A A A A C C A G A A A G T T A A C T G G T A A G T T A G T C T T T T T G T C T T T A T T T C A G G T
 C C A T G G G T G C T G C T T T A C A C T G T T G G G G A C C T A A T T G C T A C T G T G T G A A G C T G C T G
 C T G C T A C T G G A T T T T C A G T A G C T G A A A T T G C T G C T G G A G A G G C C G C T G C T G C A A T T G A A G
 T G C A A C T T G C A T C T G T T G C T A C T G T T G A A G G C C T A A C A C C T C T G A G G C A A T T G C T G C T A
 T A G G C C T C A C T C C A C A G G C C T A T G C T G T G A T A T C T G G G G C T C C T G C T G C T A T A G C T G G A T
 T T G C A G C T T T A C T G C A A A C T G T G A C T G T G T G A G C G C T G T T G C T C A A G T G G G G T A T A G A T
 T T T T A G T G A C T G G G A T C A C A A A G T T T C T A C T G T T G G T T T A T A T C A A C A A C A G G A A T G G
 C T G T A G A T T T G T A T A G G C C A G A T G A T T A C T A T G A T A T T T T A T T T C C T G G A G T A C A A A C C T
 T T G T T C A C A G T G T T C A G T A C T T T G A C C C A G A C A T T G G G G T C C A A C A C A C T T T T A A T G C C A
 T T T C T C A A G C T T T T G G C G T G T A A T A C A A A T G A C A T T C C T A G G C T C A C C T C A C A G G A G C
 T T G A A A G A A G A A C C C A A A G A T A T T A A G G G C A C A G T T T G C A A G G T T T A A A G G A A A C T A
 C T T G G A C A G T A A T T A A T G C T C C T G T T A A T T G G T A T A A C T C T T A C A A G A T T A C T A C T C T A
 C T T G T C T C C A A T T A G G C C T A C A A T G G T G A G A C A A G T A G C C A A C A G G G A A G G G T T G C A A A
 T A T C A T T T G G G C C A C A C C T A T G A T A A T A T T G A T G A A G C A G A C A G T A T T C A G C A A G T A A C T G
 A G A G G T G G G A A G C T C A A A G C C A A A G T C C T A A T G T G C A G T C A G G T G A A T T A T T G A A A A T
 T T G A G G C T C C T G G T G G T G C A A A T C A A A G A A C T G C T C C A G T G G A T G T T G C C T T A C T T C
 T A G G C C T G T A C G G A A G T T A C T T C T G C T C T A A A A G C T T A T G A A G A T G G C C C A A C A A A A
 A G A A A A G G A A G T T G T C A G G G G C A S C T C C C A A A A A A C C A A A G G A A C A G T G C A A G T G C C A
 A A G C T C G T C A T A A A G G A A G A T A G A A G T T C T A G G A G T T A A A C T G G A G T A G A C A G C T T C
 A C T G A G G T G G A G T G C T T T T A A A T C C T C A A A T G G G C A A T C C T G A T G A A C A T C A A A A G G C
 T T A A G T A A A A G C T T A G C A G C T G A A A A C A G T T T A C A G A T G A C T C C A G A C A A A G A C A A
 C T G C C T T G C T A C A G T G T G C T A G A A T T C C T T T G C C T A A T T A A T G A G G A C T T A A C C T G T
 G G A A A T A T T T T G A T G T G G G A A G C T G T T A A A A C T G A G G T T A T T G G G G T A A C T G C T
 A T G T T A A A C T T G C A T T C A G G G A C A C A A A A C T C A T G A A A T G G T G C T G G A A A C C C A T T
 C A A G G G T C A A A T T T C A T T T T T T G C T G T T G G T G G G G A A C C T T T G A G C T G C A G G G T G T G
 T T A G C A A A C T A C A G G A C C A A A T A T C C T G C T A A A C C T G T A A C C C A A A A A A T G C T A C A G T T
 G A C A G T C A G C A G A T G A A C A C T G A C A C A A G C T G T T T T G G A T A A G A T A A T G C T A T C C A
 G T G G A G T G C T G G G T T C C T G A T C C A A G T A A A A T G A A A A C T A G A T A T T T G G A A C C T A C
 A C A G G T G G G G A A A T G T G C C T C C T G T T T T G C A C A T T A C T A A C A C A G C A A C C A C A G T G C T T
 C T T G A T G A G C A G G G T G T T G G G C C T T G T G C A A A G C T G A C A G C T T G T A T G T T C T G C T G T T
 G A C A T T T G T G G G C T G T T A C C A A C A C T T C T G G A A C A C A G C A G T G G A A G G A C T T C C C A G A
 T A T T T T A A A T A C C C T T A G A A A G C G G T C T G T G A A A A C C C C T A C C A A T T T C C T T T T G
 T T A A G T G A C C T A A T T A A C A G G A G G A C A C A G A G G G T G A T G G B C A G C C T A T G A T T G G A A T G
 T C C T C T C A A G T A G A G G A G G T A G G G T T T A T G A G G A C A C A G A G A G C T T C C T G G G A T C C A
 G A C A T G A T A A G A T A C A T T G A T G A G T T T G G A C A A A C C A C A C T A G A A T G C A G T A A A A A
 T G C T T T A T T T G T G A A A T T T G T A T G C T A T T G C T T T A T T G T A A C C A T T A A A G C T G C A A T
 A A C A A G T T ... 3'

Fig. 3 Nucleotide sequence of the late region. Only the strand with the same polarity as late mRNA is shown (in a 5' to 3' orientation). The start and endpoint are the same as in Fig. 2. The cleavage sites of *Hind*II and III and *Eco*RI restriction enzymes are shown (see also Fig. 1). The presumed initiation codons for *VP*₂, *VP*₃ and *VP*₁ are boxed, as well as the termination codons for *VP*₂/*VP*₃ and for *VP*₁. The reading frame in the translated region is indicated by dots (note that the last part of the *VP*₂/*VP*₃ gene overlaps the beginning of the *VP*₁ gene).

region is entered (this is evident from a close inspection of the sequence in Fig. 2). Hence, this explanation implies that about 300 amino acids of T must be coded for by the *Hind* A fragment; about 100 amino acids may be shared with t and at most 188 may be coded for by the region before the junction to *Hind* H (map position of 0.534–0.426). This would mean that translation must indeed resume rather soon after nucleotide 672.

Although the T antigen interacts specifically with SV40 DNA^{39,40}, it is not particularly basic. In certain areas S-containing amino acids are rather frequent^{8,24} and, in general, many hydrophobic clusters are present. Particularly remarkable is the high proline content of the carboxy-terminal end: six out of ten residues are proline²². The latter aspect is confirmed by direct analysis of an adenovirus–SV40 fusion protein, in which the carboxyterminal sequence is derived from SV40 T antigen⁴¹.

T is synthesised from a 19S mRNA^{32,33}. As discussed above, the protein is coded for by two non-contiguous regions on the genome. In view of well-documented evidence from adenovirus mRNA and SV40 late mRNA^{6,42–50}, it is likely that the transposition or splicing-out of the inserted genetic information occurs at the mRNA level. Berk and Sharp⁵¹ have provided direct evidence for an early SV40 messenger which corresponds approximately to the region 0.67–0.60 linked to 0.54–0.14 and is estimated to be 2,230 nucleotides long. It may be noted, however, that for the late SV40 messengers the splicing removes segments before the structural part of the gene, while in this early mRNA, the crossovers are in translated regions. We know that the segment deleted from late mRNA is present in (most of) the nuclear RNA, and hence that splicing in this case is a post-transcriptional event (our unpublished results). Whether this is also true for the early mRNA has not yet been established.

t is synthesised from an mRNA which sediments slightly faster than the mRNA coding for T (ref. 33). According to the interpretation discussed above, only about the first quarter of the RNA molecule would be translated and the remainder would constitute a link with the region beyond nucleotide 2,552 (Fig. 2) where there are presumably essential signals for the termination of transcription, processing, polyadenylation and/or transport (Fig. 1). According to recent evidence, even t mRNA is subject to splicing; a small segment, perhaps about 50 nucleotides, is removed from the region around 0.55–0.54 (ref. 51). It is noteworthy that this corresponds to a region which is unusually AT-rich: the 51 base pairs between nucleotides 591 and 641 are 82.4% AT and a middle segment of 18 base pairs is exclusively AT. Also the region following the common leader sequence of the two (or three) late SV40 mRNAs (nucleotide 509 to 535 in Fig. 3) is remarkably AT-rich. This feature could perhaps have a role in splicing.

The 5'-end of the early mRNA has been reported to map around position 0.67 (ref. 52), but the exact location of the mRNA start is not known. The 3'-end was found to extend to about position 0.16 (ref. 53). This corresponds almost exactly to the termination codon of VP₁ (nucleotide 2,574 in Fig. 3). The signal AAUAAA is present in all eukaryotic mRNAs as well as in encephalomyocarditis virus RNA, 10 to 20 nucleotides before the poly(A) tail^{54–56}. It is present twice in SV40 early mRNA and its complement can be seen at nucleotide 2,618–2,613 and again at nucleotide 2,589–2,584 in Fig. 3. Detailed comparison of the viral, cytoplasmic mRNAs with the DNA sequence indicates that the 3'-terminal, untranslated regions of the early and late mRNAs overlap for a distance of about 80–100 nucleotides^{22,53}. As was found for parts of the regions spliced out of the early and late transcripts, the untranslated segment between the termination codons of the T antigen gene and the VP₁ gene is rather AT-rich (74.5% compared with 59.5% for the total SV40 genome).

The late region

Late transcription starts concomitantly with or following SV40 DNA replication, which itself requires a functional early gene A product. No late transcription occurs when DNA replication is blocked (as in non-permissive cells or after the addition of appropriate inhibitors). The 5'-end of late mRNA maps around 0.72, but its precise position is unknown^{13,14,32}; the heteropolymeric 3'-end of the cytoplasmic mRNA to which the poly(A) tail is added, corresponds almost exactly to the *Hind* G–*Hind* B junction (see below; refs 50, 57 and Fig. 1).

The late region is known to code for only three structural proteins, VP₁, VP₂ and VP₃. The corresponding genes have been approximately localised on the genome by mapping temperature-sensitive mutations and DNA deletions which affect a particular protein^{16,17,25,31,58}. All tryptic peptides derived from VP₃ are also present in a fingerprint of VP₂^{59,60} and since deletions at the beginning of the VP₂ gene do not affect the size of VP₃, it is generally believed that translation of the VP₃ gene is initiated inside the VP₂ gene and from there on is read in the same reading frame^{31,60}.

The nucleotide sequence of the entire late region is shown in Fig. 3. Segments have been described before in detail: *Hind* C⁶¹, *Hind* L and *Hind* M⁶², *Hind* D^{63,64}, *Hind* E⁶⁵, *Hind* K^{7,66}, *Hind* F^{67,68}, *Hind* J⁶⁹ and *Hind* G⁷⁰ (see ref. 10 for parallel results obtained by S. Weissman and colleagues; *Hind* M and a segment around the *Hind* C/A junction have been independently sequenced by R. Wu and coworkers^{71,72}).

The origin of DNA replication is around 0.67 map units; Subramanian *et al.*¹¹ pointed out that this region contains a remarkable palindromic sequence of 27 base pairs. This

Fig. 4 Amino acid sequence of the early region of SV40 DNA. The sequence in the top part corresponds to t antigen (174 amino acids). T antigen starts at the same position and shares an estimated 100 amino acids with t; the lower part gives the maximal sequence which can follow the common N-terminal segment. For reasons discussed in the text, it is likely that indeed most of this coding potential is actually used.

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Met-Asp-Lys-Val-Leu-Asn-Arg-Glu-Glu-Ser-Leu-Gln-Leu-Met-Asp-Leu-Leu-Gly-Leu-Glu- 20
Arg-Ser-Ala-Trp-Gly-Asn-Ile-Pro-Leu-Met-Arg-Lys-Ala-Tyr-Leu-Lys-Lys-Cys-Lys-Glu- 40
Phe-His-Pro-Asp-Lys-Gly-Gly-Asp-Glu-Glu-Lys-Met-Lys-Lys-Met-Asn-Thr-Leu-Tyr-Lys- 60
Lys-Met-Glu-Asp-Gly-Lys-Tyr-Ala-His-Gln-Pro-Asp-Phe-Gly-Gly-Phe-Trp-Asp-Ala- 80
Thr-Glu-Val-Phe-Ala-Ser-Ser-Leu-Asn-Pro-Gly-Val-Asp-Ala-Met-Tyr-Cys-Lys-Gln-Trp-100
Pro-Glu-Cys-Ala-Lys-Lys-Met-Ser-Ala-Asn-Cys-Ile-Cys-Leu-Leu-Cys-Leu-Leu-Arg-Met-120
Lys-His-Glu-Asn-Arg-Lys-Leu-Tyr-Arg-Lys-Asp-Pro-Leu-Val-Trp-Val-Asp-Cys-Tyr-Cys-140
Phe-Asp-Cys-Phe-Arg-Met-Trp-Phe-Gly-Leu-Asp-Leu-Cys-Glu-Gly-Thr-Leu-Leu-Leu-Trp-160
Cys-Asp-Ile-Ile-Gly-Lys-Thr-Thr-Thr-Arg-Asp-Leu-Lys-Leu-174

Ile-Pro-Thr-Tyr-Gly-Thr-Asp-Glu-Trp-Glu-Gln-Trp-Trp-Asn-Ala-Phe-Asn-Glu-Glu-Asn- 20
Leu-Phe-Cys-Ser-Glu-Glu-Met-Pro-Ser-Ser-Asp-Asp-Glu-Ala-Thr-Ala-Asp-Ser-Gln-His- 40
Ser-Thr-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-Glu-Asp-Pro-Lys-Asp-Phe-Pro-Ser-Glu-Leu- 60
Leu-Ser-Phe-Leu-Ser-His-Ala-Val-Phe-Ser-Asn-Arg-Thr-Leu-Ala-Cys-Phe-Ala-Ile-Tyr- 80
Thr-Thr-Lys-Glu-Lys-Ala-Ala-Leu-Leu-Tyr-Lys-Lys-Ile-Ile-Glu-Lys-Tyr-Ser-Val-Thr-100
Phe-Ile-Ser-Arg-His-Asn-Ser-Tyr-Asn-His-Asn-Ile-Leu-Phe-Phe-Phe-Thr-Pro-His-Arg-120
His-Arg-Val-Ser-Ala-Ile-Asn-Asn-Tyr-Ala-Gln-Lys-Leu-Cys-Thr-Phe-Ser-Phe-Leu-Ile-140
Cys-Lys-Gly-Val-Asn-Lys-Glu-Tyr-Leu-Met-Tyr-Ser-Ala-Leu-Thr-Arg-Asp-Pro-Glu-Ser-160
Val-Ile-Glu-Glu-Ser-Leu-Pro-Gly-Gly-Leu-Lys-Glu-His-Asp-Phe-Asn-Pro-Glu-Ala-180
Glu-Glu-Thr-Lys-Gln-Val-Ser-Trp-Lys-Leu-Val-Thr-Glu-Tyr-Ala-Met-Glu-Thr-Lys-Cys-200
Asp-Asp-Val-Leu-Leu-Leu-Gly-Met-Tyr-Leu-Glu-Phe-Glu-Thr-Tyr-Ser-Phe-Glu-Met-Cys-220
Leu-Lys-Cys-Ile-Lys-Lys-Glu-Gln-Pro-Ser-His-Tyr-Lys-Tyr-His-Glu-Lys-His-Tyr-Ala-240
Asn-Ala-Ala-Ile-Phe-Ala-Asp-Ser-Lys-Asn-Gln-Lys-Thr-Ile-Cys-Gln-Gln-Ala-Val-Asp-260
Thr-Val-Leu-Ala-Lys-Lys-Arg-Val-Ser-Leu-Gln-Leu-Thr-Arg-Glu-Glu-Met-Leu-Thr-280
Asn-Arg-Phe-Asn-Asp-Leu-Leu-Asp-Arg-Met-Asp-Ile-Met-Phe-Gly-Ser-Thr-Gly-Ser-Ala-300
Asp-Ile-Glu-Glu-Trp-Met-Ala-Gly-Val-Ala-Trp-Leu-His-Cys-Leu-Leu-Pro-Lys-Met-Asp-320
Ser-Val-Leu-Tyr-Asp-Phe-Leu-Lys-Lys-Met-Val-Tyr-Asn-Ile-Pro-Lys-Lys-Arg-Tyr-Trp-340
Leu-Phe-Lys-Gly-Pro-Ile-Asp-Ser-Gly-Lys-Thr-Thr-Leu-Ala-Ala-Leu-Leu-Glu-Leu-360
Cys-Gly-Gly-Lys-Ala-Leu-Asn-Val-Asn-Leu-Pro-Leu-Asp-Arg-Leu-Asn-Phe-Glu-Leu-Gly-380
Val-Ala-Ile-Asp-Gln-Phe-Leu-Val-Val-Phe-Glu-Asp-Val-Lys-Gly-Thr-Gly-Gly-Glu-Sep-400
Arg-Asp-Leu-Pro-Ser-Gly-Gln-Gly-Ile-Asn-Asn-Leu-Asp-Asn-Leu-Arg-Asp-Tyr-Leu-Asp-420
Gly-Ser-Val-Lys-Val-Asn-Leu-Glu-Lys-Lys-His-Leu-Asn-Lys-Arg-Thr-Gln-Ile-Phe-Pro-440
Pro-Gly-Ile-Val-Thr-Met-Asn-Glu-Tyr-Ser-Val-Pro-Lys-Thr-Leu-Gln-Ala-Arg-Phe-Val-460
Lys-Gln-Ile-Asp-Phe-Arg-Pro-Lys-Asp-Tyr-Leu-Lys-His-Cys-Leu-Glu-Arg-Ser-Glu-Phe-480
Leu-Leu-Glu-Lys-Arg-Ile-Ile-Gln-Ser-Gly-Ile-Ala-Leu-Leu-Leu-Ile-Leu-Ile-Trp-Tyr-500
Arg-Pro-Val-Ala-Glu-Phe-Ala-Gln-Ser-Ile-Gln-Ser-Arg-Ile-Val-Glu-Trp-Lys-Glu-Arg-520
Leu-Asp-Ser-Gln-Ser-Gln-Gly-Ser-Phe-Gln-Ala-Pro-Gln-Ser-Gln-Ser-Leu-Val-His-Asp-540
Ile-Gly-Val-Leu-Asp-Trp-Leu-Arg-Asn-Ser-Asp-Asp-Asp-Glu-Asp-Ser-Gln-Glu-Asn-560
Ala-Asp-Lys-Asn-Glu-Asp-Gly-Gly-Glu-Lys-Asn-Met-Glu-Asp-Ser-Gly-His-Glu-Thr-Gly-580
Ile-Asp-Ser-Gln-Ser-Gln-Gly-Ser-Phe-Gln-Ala-Pro-Gln-Ser-Gln-Ser-Leu-Val-His-Asp-600
His-Asn-Gln-Pro-Tyr-His-Ile-Cys-Arg-Gly-Phe-Thr-Cys-Phe-Lys-Lys-Pro-Pro-Thr-Pro-620
Pro-Pro-Glu-Pro-Glu-Thr-626

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segment is also rather well conserved in polyoma DNA (refs 73, 74; B. Griffin, personal communication). The first structural gene, which codes for VP₂, presumably starts at position 543 (Fig. 3). This has not been firmly established by correlation with protein sequence data but is supported by various arguments⁶³. As discussed above, the first initiating codon in the early region of the genome is at position 80 (Fig. 2). Hence, in between these divergently directed structural genes there is a 622 base pair (542+79+1) DNA segment which contains information not only for initiation of DNA replication, but presumably also for early transcription, late transcription, for ribosome binding and initiation of translation, and for the processing of mRNAs, as discussed below (some of these biological signals may of course overlap each other or part of the structural genes). This DNA sequence contains many remarkable features, such as palindromes, long repeats, so-called 'true' palindromes, blocks with an exceptionally high AT-content which are moreover asymmetric (that is T residues in one strand and A residues in the other) and unusual alternations of AT-rich and GC-rich blocks^{11, 61-63, 72, 75}. The biological significance of these features is largely unknown, but at least some of these sequences can be deleted. In particular, a segment of about 90 nucleotides from map position 0.675 to 0.692 is redundant⁷⁶. Other deletions in this area are still viable but result in a decreased plaque size and/or burst size^{77, 78}.

We believe that the VP₂ gene starts at position 543 and the VP₃ gene at position 897 and that both end with a UAA at 1,599 (refs 63-66). Initiation elsewhere would either involve GUG as a start codon (which is unlikely) or result in an unrealistic polypeptide size. VP₂ contains 352 amino acids before processing and VP₃, 234 amino acids. The corresponding molecular weights of 38,533 and 26,967, respectively, are in reasonable agreement with direct size estimates based on the electrophoretic mobility of these proteins in SDS-polyacrylamide gels (see ref. 79 for example). The amino acid sequence has been published^{7, 63-66} and can be deduced from the information given in Fig. 3. It should be noted that this sequence refers to the primary translation product; that may be subsequently processed and is presumably blocked at the N-terminus⁹⁸. The composition deduced corresponds moderately well with the directly determined values⁸¹ and in particular shows the absence of cysteine residues in VP₃ (ref. 82). The aminoterminal sequence of VP₂ is exceptionally rich in alanine and is very hydrophobic; it contains no basic residue in the first 98 amino acids. Conceivably, it may be involved in an interaction with membranes. The carboxy-terminus of VP₂ and VP₃ is, however, unusually basic; 15 of the last 34 residues are either lysine or arginine. This basic tail may perhaps anchor the protein to the DNA^{83, 84}. It is of interest that the VP₂/VP₃ gene overlaps the VP₁ gene over a distance of 122 nucleotides⁶⁵. Since the common segment is read in two different translation frames, it may be less malleable evolutionarily and this may be why it is precisely this region which cross-hybridises with the polyoma genome⁸⁵.

Lazarides, Files and Weber⁸⁰ determined the first seven amino acids of VP₁. With this information we could establish that the codon for the N-terminal alanine starts at position 1,489 (refs 7, 86). It is preceded by an AUG codon, but another AUG appears two codons before. Recent *in vitro* translation experiments suggest that the latter is used for initiation (A. Mellor, R. Hewick, and A. E. Smith, personal communication). This would mean that a Met-Lys-Met sequence is subsequently processed away. The VP₁ polypeptide is 361 amino acids long and its entire sequence has been published^{6, 87}. It has a molecular weight of 39,708 which is considerably less than the 42,000 to 47,000 estimated by its mobility in SDS gels (see ref. 79

for example). This discrepancy may possibly be related to the high proline content. The VP₁ gene is terminated by a single UGA signal starting at position 2,572 in *Hind* G.

Once the lytic infection is fully under way, the amount of late virus-specific RNA in both the cytoplasm and the nucleus is 10 to 20-fold higher than the RNA derived from the early region. Two late poly(A)-containing mRNAs—19S and 16S—can readily be resolved, coding for VP₂ and VP₁ respectively. In the case of polyoma it is known that VP₃ is made on a separate 18S mRNA (ref. 88; B. Kamen, personal communication; T. Hunter, personal communication), and the same may be true for SV40. The bulk of the 16S mRNA hybridises to the restriction fragments *Hind* K, F, J and G^{89, 90}, which, as we have seen, contain the structural information for VP₁. Late 19S mRNA hybridises, in addition, to the *Hind* fragments D and E, which together with *Hind* K, contain the VP₂ gene⁹¹. Obviously, 19S mRNA (and the putative 18S mRNA) carries the information for making VP₁ but does not express it (Fig. 1). The initiating AUG for VP₁ is possibly too far away from the capped 5'-terminus, or is perhaps buried in a secondary structure; a hairpin model for this region has been proposed⁸⁵.

Zain *et al.*⁸⁷ have reported that the T₁-oligonucleotide (G)UUAACAACAACAAUUG, which corresponds to the junction of the fragments *Hind* G-*Hind* B, is no longer present in the late mRNAs, although all preceding T₁-oligonucleotides can be identified. We have confirmed this and found that the 3'-end of both late mRNAs is in the form (G)UU(AAC)1-3 poly(A). Counting from the U-A junction (the last nucleotide in Fig. 3), the 3'-untranslated sequence of 16S mRNA is 78 nucleotides (including the terminator UGA). It contains the sequence AAUAAA at a distance of 12-7 nucleotides from the U-A junction (this common signal present in 3'-untranslated regions was discussed above for the early mRNA). Under certain conditions, single-stranded SV40 DNA forms hairpins which can be fixed and visualised by electron microscopy^{92, 93}. One hairpin occurs around map position 0.17 and may correspond to a hairpin structure which can be proposed for the 3'-untranslated segment⁷⁰. It is possible that in the nucleus the transcription runs somewhat further and is terminated in *Hind* B^{14, 55}. This sequence would correspond to anti-early mRNA (see Fig. 2).

The 5'-end of SV40 mRNA is a 7-methyl G cap⁹⁴⁻⁹⁶. Further investigation has revealed that both 19S and 16S late mRNAs start with either 7-methyl Gppp 6,2'-dimethyl ApU or 7-methyl Gppp 6,2'-dimethyl Ap 2'-methyl UpU, the latter being relatively enriched in the 16S mRNA⁹⁴. It remains to be established whether both types of cap structure come from the same place on the genome and whether they are directly derived from the 5'-end of the primary transcript.

A number of laboratories have recently reported that the 5'-leader sequence of adenovirus mRNA is coded for by several non-contiguous regions which precede and are separated from the structural body of the gene by a considerable distance⁴²⁻⁴⁶. A similar observation for SV40 was made by hybridisation of purified late mRNA to *Eco*RI-digested DNA or to appropriate restriction fragments^{47, 48}. By comparison of the fingerprints of the late mRNAs with the known DNA sequence of the genome it was possible to conclude independently that these messengers are spliced^{6, 49, 50}. Since the T₁-oligonucleotides between positions 344 and 501 are all present in both the late 19S fingerprint and in the 16S fingerprint, the common 5'-leader sequence must be at least 161 nucleotides long and the capped oligonucleotides must be derived from residue 308 or closer to the origin. The segment spliced out of the 16S RNA must start somewhere between residues 502 to 517 and terminate in the region of residues 1,431 to 1,458, that is, at least 21 residues before the presumptive AUG initiation

	U	C	A	G	
U	Phe { 22 1 16 16 } Leu { 16 16 }	Ser { 16 4 6 }	Tyr { 16 9 1 1 } Ochre Amber	Cys { 3 4 1 10 } Opal Trp	U C A G
C	Leu { 10 3 6 6 }	Pro { 21 8 15 }	His { 6 5 26 16 }	Arg { 2 1 1 1 }	U C A G
A	Ile { 23 8 2+11 }	Thr { 25 15 16 }	Asn { 19 11 28 8 }	Ser { 16 6 15 17 }	U C A G
G	Val { 24 1 11 18 }	Ala { 45 6 12 }	Asp { 18 17 22 19 }	Gly { 12 7 19 15 }	U C A G

Fig. 5 Codons used in the SV40 late genes (VP_1 and VP_2). The numbers refer to the frequency with which each triplet is used. The data for VP_1 and VP_2 are combined (a separate table for VP_1 has previously been published in ref. 6). The codeword table for the early genes is relatively similar.

codon of VP_1 (ref. 50). Since the T_1 -oligonucleotides between residues 513 and 538 are missing from the late 19S fingerprint, a smaller fragment of 10 to 41 nucleotides must be spliced out of the 19S mRNA. The reason for this splicing and how it comes about is not known.

General characteristics and use of code words

SV40 DNA is 5,224 nucleotides long (2,574+2,649+1). At least 15.2% of this genetic material does not code directly for protein synthesis (this is a minimum percentage as the exact size of the region spliced out of the T gene is not known). A segment of 122 nucleotides (2.3% of the total genome) located in the late region, is read in two different frames and fulfils a dual function by coding for parts of three different proteins, VP_3/VP_2 and VP_1 . A considerable portion of the genome directs the synthesis of two different proteins albeit read in the same frame (t and T antigens; VP_3 and VP_2).

Earlier studies had indicated⁸⁷, and confirmed^{8,87,98}, that in SV40 DNA, as in all vertebrate DNA, the dinucleotide sequence CG is very infrequent. It is of interest to note, however, that this drastic deficiency applies mainly to the translated regions and much less to the control region between the initiation codons of the early and late genes (map position 0.648 to 0.767, corresponding to nucleotide 80 in Fig. 2 and nucleotide 543 in Fig. 3). In this control region, CG occurs 18 times relative to an expected value of 36 to 37, whereas in the remainder of the genome it occurs only 9 times (expected 183). Our previous conclusions on the non-random utilisation of codons in SV40 DNA^{8,7} become strengthened now that the total sequence is known. A codeword table for the late region is given in Fig. 5, but we stress that approximately the same relative distribution is valid for the early genes. Particularly striking is the deficiency of codons containing the sequence CG: the codons of the type NCG for serine, proline, threonine, or alanine are not used at all and only 3 out of 35 codons for arginine are of the CGN type (Fig. 5). Of the 97 codons ending with C, only 4 are followed by a code word beginning with G (1 occurs in the overlap region between the VP_2/VP_3 gene and the VP_1 gene). In the translated early region, the sequence CG occurs only 3 times.

Another striking feature of the codeword table is the low frequency of codons of the type NUC; in fact, the codon for isoleucine, AUC does not occur at all in the whole genome. Also statistically highly significant is the fact that codons of the type UUPu are preferred over CUN for leucine, and the codon AAA is preferred over AAG for lysine⁶. Although relative codon utilisation is remarkably consistent over the entire SV40 genome, it cannot

reflect a general property of higher animal cells. Indeed, in other systems, highly non-random, but quite different patterns of selective codon utilisation are encountered (ref. 56 and references therein). Presumably, as in prokaryotes⁹⁹, various factors such as the tRNA composition, modulation effects, codon-anticodon interaction energy, and structural requirements of the mRNA influence the use made of the degeneracy of the code.

The elucidation of the total SV40 DNA sequence has revealed the complex and efficient organisation of the genes and has allowed us to deduce the almost complete amino acid sequence of all five SV40-coded proteins. Further study of the relationship between DNA structure and function may reveal any additional genetic information. The more difficult task remains, however, of explaining the complex biological functions and effects of this oncogenic virus in terms of the primary structure of its gene products.

We thank our colleagues who helped us at different stages of this project with advice, information and materials, and particularly Drs W. Gilbert, B. Hirt, K. Kleppe, A. Maxam, P. May and D. Nathans. Drs K. Danna, E. Soeda, R. Thijs and R. Yang contributed to earlier parts of this research project. We thank José Van der Heyden for assistance. Our research was supported by a grant from the Kankerfonds of the Algemene Spaar-en Lijfrentekas (ASLK) of Belgium. R.C. and G.H. hold fellowships from the NFWO.

Received 23 January; accepted 22 March 1978.

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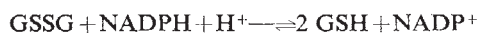
The structure of the flavoenzyme glutathione reductase

G. E. Schulz, R. H. Schirmer, W. Sachsenheimer & E. F. Pai

Max-Planck-Institut für Medizinische Forschung, Jahnstrasse 29, 6900 Heidelberg, FRG

The three-dimensional structure of the dimeric flavoenzyme glutathione reductase from human erythrocytes has been elucidated by an X-ray diffraction analysis at 0.3 nm resolution. The polypeptide chain has been traced, and the binding positions of FAD, NADP and glutathione have been determined. A mechanism for the electron transfer is discussed.

GLUTATHIONE reductase (EC 1.6.4.2), a ubiquitous FAD-containing enzyme, uses NADPH to reduce oxidised glutathione (GSSG):



The enzyme's general function is to maintain a high level of reduced glutathione in the cytosol¹, but it may have additional roles, for example, in the cell division cycle² and in stress adaptation on a cellular level³. The enzyme is closely related to other disulphide reductases such as lipamide dehydrogenase and thioredoxin reductase^{1,4}. In our analysis we used glutathione reductase from human erythrocytes, which is a dimer of two identical subunits⁵ each of molecular weight of 50,000 (refs 6, 7).

X-ray diffraction analysis

Human erythrocyte glutathione reductase crystallises in space group B2 with parameters $a = 11.97$ nm, $b = 8.46$ nm, $c = 6.34$ nm, $\gamma = 58.6^\circ$. An X-ray analysis of these crystals at a resolution of 0.6 nm has been reported earlier⁸. Using the isomorphous replacement method, the analysis has now been extended to the 11,300 independent reflections corresponding to a resolution of 0.3 nm.

The reflection intensities were determined on an automated diffractometer (P2₁, Syntex) following a previously described procedure⁸. For our measurements the beam geometry was modified and the goniometer was thermally isolated and cooled to $7 \pm 3^\circ\text{C}$. Intensity data were collected in seven shells of about 1,700 reflections each, which were radially ordered in reciprocal space. For each data set we made a separate measurement of about 350 strong reflections that overlapped with each shell in about 50 reflections. Using these overlaps, the shells were put on a common scale. Subsequently, this scale was adjusted to an approximately absolute level on the basis of a Wilson plot⁹.

To improve accuracy, the easily available native protein data were collected four times and averaged. Cooling enabled us to measure each heavy atom derivative data set with one crystal. Thus, errors due to variations in derivative production were eliminated. The impact of radiation damage was minimised by measuring the shells from smaller spacings to larger ones, that is, centripetally in reciprocal space. In no case did the radiation damage exceed 20%.

For the analysis we used five heavy atom derivatives (Table 1). All heavy atom positions were located with difference Fourier techniques based on the heavy atom sites known from the analysis at 0.6 nm resolution⁸. The heavy atom parameters were then refined following a procedure¹⁰ that alternates between evaluation of 'best' phases and parameter shifts.

The result of this refinement is given in Table 1. There are five recurring sites, I, II, III, IV, V, and two further ones. As the derivatives show different occupancies of these sites, the heavy atom structure factors of the respective derivatives become largely independent of each other.

The z -values of sites I, III and IV are close to 0.0 and 0.5. In space group B2 the heavy atom structure factors based on such sites have phase angles 0 or π . Consequently, in the phase

calculations¹⁰, the 'best' protein phases are also restricted to these values. The first four derivatives of Table 1 show only minor sites deviating appreciably from these special positions, but the last derivative is quite different and considerably improves the phases.

The R_c -factors, the F_H/E -values, $\Delta\alpha$ and the mean figure of merit given in Table 1 all indicate that the phases are of reasonable quality. With all $|\alpha_F - \alpha_H|$ -values close to the expected value of 90°, the derivative scaling factors are confirmed, and no major errors in the electron density map need be anticipated near the heavy atom positions¹¹.

The binding positions of NADP and oxidised glutathione had been identified previously⁸. In order to elucidate their conformations more accurately we took intensity data from a crystal soaked for 10 d at 4°C in a solution containing 10 mM NADP and 10 mM oxidised glutathione. The density distribution of the bound compounds was determined with a difference Fourier synthesis¹².

Electron density map

The electron density distribution was calculated by a Fourier synthesis of the 'best' protein structure factors¹³. The map was computed in sections 0.99 Å (= $c/64$) apart perpendicular to the c -axis and drawn on perspex sheets on a scale of 1 Å = 3 mm. The resulting map was very practical for chain tracing as well as for locating α -atoms.

Although the amino acid sequence of glutathione reductase is for the most part unknown¹⁴, the chain could be traced unambiguously. Only quite large changes in the underlying density map would allow different interpretations. The prosthetic group FAD could be recognised without problem; its pyrophosphate moiety has the highest density in the map; also, the 'glupo-fragment'¹⁴ which contains a redox-active disulphide¹ could be located. At 0.6 nm resolution the separation of subunits had been partly ambiguous⁸. We now realize

that about 35 residues had been assigned to the wrong subunit.

When locating C_α -atoms, we found 462 residues per subunit, which agrees well with the number of 463 as derived from amino acid composition and molecular weight studies⁶. The nature of each side chain was guessed from the density map. With respect to the most easily recognisable side chains (Trp, Tyr, Phe, His, Cys, Met) we assigned (3, 11, 12, 14, 10, 10) residues, whereas (4, 12, 13, 15, 10, 16) residues had been determined by amino acid analysis⁶. Except for Met the correlation is rather good, which to some extent demonstrates the quality of the map.

Description of the molecule

A stereo plot of the C_α -atoms of one subunit is given in Fig. 1. As a guide through the chain fold a sketch is depicted in Fig. 2. The enzyme subunit consists of three structural domains⁸; their location in the dimeric enzyme and their relation to the binding positions of FAD, NADP, and GSSG are shown in Fig. 3. The FAD- and NADP-(binding) domains are globular, whereas the interface-domain is somewhat flat. As their boundaries are clearly visible scissures in the overall density distribution, the domains are rather well defined entities.

The domains are not completely separated along the linear polypeptide chain¹⁵ as is the case, for instance, with the immunoglobulins. The NADP- and the interface-domain are formed by continuous sections of chain, but the FAD-domain consists of two noncontinuous parts of the chain (Fig. 3), namely residues 1–139 and 273–344. Also part of the FAD-domain makes an extended loop as two antiparallel helices (residues 45–104) to the molecular diad (Figs 1 and 2) forming a contact with its counterpart in the other subunit. Between this contact and the large contact area of the interface domains there exists an isolated hole of about 0.6 nm diameter at the molecular diad.

The molecule is rich in secondary structures; 31% of all residues are in helical conformation and 28% are in β -sheets.

Table 1 Results of the isomorphous replacement method

Heavy atom compound	Concentration applied (mM)	Soaking time (d)	R_F^* (%)	$R_C^\dagger$ (%)	$F_H/E^\ddagger$	$ \alpha_F - \alpha_H ^\S$ (degree)	$K $	$\beta $	Site	$x^\P $	$y^\P $	$z^\P $	$A^\P $	$B^\P $
(fractional coordinates)														
H[AuCl ₄]	2	25	15	42	2.2	87.3	0.970	1.0	I	0.039	0.481	0.484	48.2	6
									II	0.481	0.419	0.302	23.0	64
									III	0.247	0.178	0.499	10.6	13
									IV	0.374	0.211	0.731	23.4	66
CH ₃ ·HgNO ₃	0.15	0.9	12	50	2.1	87.1	0.957	1.4	II	0.450	0.403	0.301	19.4	15
									III	0.247	0.178	0.501	42.5	8
									IV	0.250	0.235	0.006	11.2	5
									V	0.468	0.327	0.591	12.4	11
H[AuCl ₄] and CH ₃ ·HgNO ₃	2 0.05	15 0.8	17	45	2.3	86.7	0.914	1.5	I	0.039	0.481	0.483	42.0	5
									II	0.464	0.404	0.302	21.9	55
									III	0.247	0.178	0.500	34.1	10
									IV	0.247	0.234	0.008	20.8	12
Hg(C ₆ H ₅) ₂	saturated	1.8	13	50	2.1	87.6	0.938	1.1	III	0.248	0.177	0.498	40.1	10
									IV	0.250	0.231	0.006	34.1	10
									V	0.471	0.327	0.599	1.8	4
K ₂ [Hg(CN) ₄]	2	2	27	58	1.8	89.3	0.877	1.1	I	0.073	0.264	0.693	62.5	7
									II	0.484	0.410	0.272	45.8	5
									III	0.250	0.175	0.498	51.9	10
									IV	0.247	0.244	0.980	54.6	13
									V	0.467	0.324	0.591	29.4	11

Mean figure of merit¹⁰ = 0.85. Mean difference between 'best' and 'most probable' phases¹⁰, $\Delta\alpha = 11^\circ$.

* Mean change of structure factor between two data sets: $R_F = 2(\Sigma |F_1| - |F_2|)/(\Sigma |F_1| + |F_2|)$.

† Centric reliability factor²⁷.

‡ Ratio between r.m.s. heavy-atom scattering amplitude and lack of closure error¹⁰.

§ Mean difference between phases of protein structure factor and heavy-atom structure factor.

|| Scaling parameters: initially all data sets were scaled to equal $\Sigma |F|$. In the refinement a scale factor $1/K \exp \beta S^2$ with $S = 2 \sin \theta / \lambda$ was allowed for each derivative set.

¶ Site parameters defining the heavy-atom structure factor by $\Sigma_n A_n \cdot \exp(-B_n S^2) \cdot \exp 2\pi i(hx_n + ky_n + lz_n)$ with the sum taken over all sites n of a derivative in the unit cell.

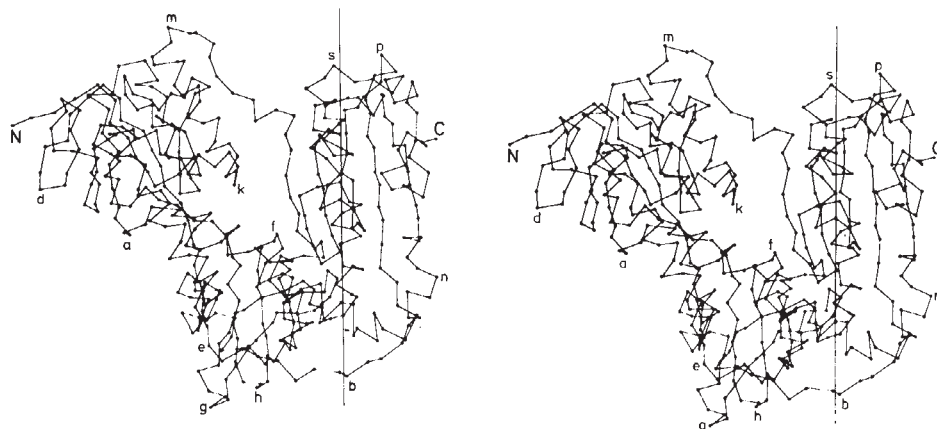
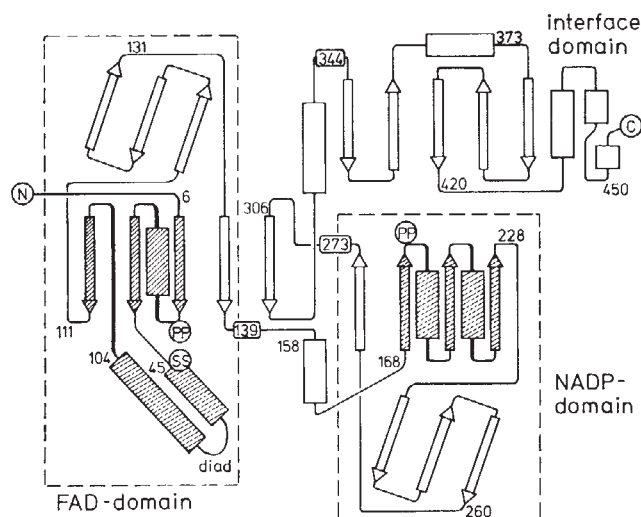


Fig. 1 Stereo drawing of the polypeptide chain of one subunit of glutathione reductase. C_{α} -positions are marked with dots. N- and C-termini as well as some residues (a = 35, b = 72, d = 126, e = 163, f = 175, g = 238, h = 260, k = 318, m = 339, n = 390, p = 409, s = 437) are inserted. The molecular diad of the dimeric enzyme is given. The subdivision of one monomer into three domains can be visualised. The NADP-domain is in the middle, the FAD- and interface-domains are at the upper left and right sides, respectively.

Many of the remaining residues form reverse turns of various types¹⁶. In each domain there is a major pleated sheet. In the FAD- and NADP-domains these sheets are of the parallel type (Fig. 2), whereas the interface-domain contains an antiparallel one. Both the FAD- and NADP-domains contain an additional small antiparallel sheet, a β -meander¹⁷, the plane of which is parallel to the central sheet.

As indicated in Figs 3 and 4, FAD and NADP are bound to their respective domains in an extended conformation reminiscent of NAD bound to dehydrogenases¹⁸. In contrast, GSSG is bound between the FAD-domain and the interface-domain of the other subunit, such that both tripeptidic halves, GS_I and GS_{II} (Fig. 4) are stretched out between subunits. The electron density map shows clearly that the active centre is located between subunits. Active centres shared by subunits were also found in glucose 6-phosphate isomerase²⁹ and to a lesser extent in D-glyceraldehyde-3-phosphate dehydrogenase^{31, 32}.

Fig. 2 Two-dimensional sketch of the chain fold of one subunit of glutathione reductase approximating the view of Fig. 1. Strands of β -sheets are given as arrows and helices as rectangles. N- and C-termini as well as some residue numbers are inserted. The three encircled residue numbers denote domain boundaries. The Rossmann-folds are hatched. If the parallel sheets are in the paper plane, the helices in both Rossmann-folds are above this plane. The binding positions of the pyrophosphate moieties of FAD and NADP at the backbone are labelled (PP). The active disulphide (SS) is at the start of a long helix. The chain segments which may have arisen by gene duplication are framed by dashed lines. In both segments the antiparallel sheet is located behind the parallel sheet, that is, below the paper plane. The relative location and orientation of parallel and antiparallel sheets are identical in both segments. Except for the excursion of residues 45–104 the segments resemble each other topologically and geometrically.



Another peculiar feature of the molecule is the close contact between the flavin ring and the chain of the interface-domain of the other subunit (Fig. 3). This contact is probably a hydrogen bond between O2 α and/or N3 of the flavin ring and the backbone at residue 450. With this ring orientation the 7 α - and 8 α -methyl groups are in a region of small density, that is the nonpolar moiety of FAD is immersed in a nonpolar environment.

Chain fold comparisons with other proteins

As glutathione reductase binds two dinucleotides, FAD and NADP, we screened the respective domains for structural features that are familiar from other nucleotide binding proteins. Each of these two domains contains a parallel sheet in its centre. In the NADP-domain, three adjacent strands of this sheet are connected through helices in such a way that a 'Rossmann-fold' is formed, a supersecondary structure occurring in most structurally known nucleotide binding proteins¹⁵. Also in the FAD-domain there are three adjacent sheet strands which are connected through helices to form a Rossmann-fold. However, there is a long extended loop (residues 45–104) containing the redox-active disulphide in one of the connections between β -strands (Fig. 2).

The most conspicuous similarity to other nucleotide binding proteins, however, is the binding mode of the dinucleotides relative to the Rossmann-folds. In the FAD- as well as in the NADP-domain, the dinucleotide is stretched across the carbonyl ends of the parallel sheet strands such that (1) the pyrophosphate moieties touch the backbone at the loop between first β -sheet strand and first helix of the Rossmann-fold, (2) the flavin and nicotinamide moieties, respectively, are located at the side of the sheet which accommodates the helices of the Rossmann-fold, and (3) the adenosine moieties bind at the other side of the sheet in a groove between this sheet and the antiparallel β -meander. The same general binding mode has been observed in other structurally known nucleotide binding proteins^{17–19}. In particular, in all observed cases, phosphoryl binding to the backbone occurs at the described position in the Rossmann-fold.

Thus, we find that both the FAD- and the NADP-domain resemble other nucleotide binding proteins^{18,19} with respect to chain fold and binding mode. This resemblance may indicate a distant evolutionary relationship.

Internal chain fold repeat

The similarities described above suggest that FAD- and NADP-domains resemble each other. Indeed, the chain folds of residues 6–139 (first chain segment of FAD-domain) and residues 168–272 (C-terminal part of the NADP-domain) are topologically equivalent and closely similar in geometry. These chain segments consist of the Rossmann-folds, the β -meanders and a 4th strand of the parallel sheets (Fig. 2). The β -meanders are included because (1) the connections of these small antiparallel sheets with the 4-stranded parallel sheets are equivalent to each other, (2) the planes of both β -meanders are parallel to the respective

4-stranded sheets, and (3) the angles between meander sheet strands and central sheet strands agree with each other. In addition to the topological similarity of the chain folds, the nucleotide binding positions are also equivalent (see above).

Thus, the resembling structures are rather complex, so that their similarity is highly significant²⁰. Consequently, they indicate an evolutionary relationship between the two domains, indicating that a primordial gene duplication accompanied or followed by gene splicing is very likely. The described similarity cannot be extended to residues 140–167 in the N-terminal part of the NADP-domain and to the second chain segment in the FAD-domain (residues 273–344). Conspicuously, these chain regions are near to and also involved in the active centre; at such specific positions variations of a basic structural motif are to be expected.

The active centre

As shown in Figs 4 and 5, at least two redox systems are involved in the transfer of reduction equivalents from NADPH to GSSG, the isoalloxazine ring of FAD and the disulphide formed by Cys-46 and Cys-41 (refs 1, 4). The 'proximal' sulphur of this disulphide (Cys-46) touches the isoalloxazine ring at or close to position 4a; the 'distal' sulphur (of Cys-41) is in van der Waals contact with the disulphide of GSSG. It is not possible that a hydride ion can be transferred directly from NADPH to either of the two cysteines, because the isoalloxazine ring forms an insurmountable barrier.

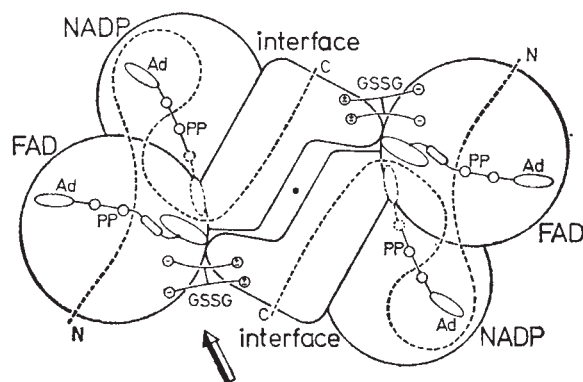


Fig. 3 Sketch of the dimeric enzyme glutathione reductase. The FAD-, NADP- and interface-domains are outlined. The approximate view of Fig. 1 is indicated by an arrow. The general course of the polypeptide chain is given by a dashed line. FAD and NADP bind to their respective domains in an extended conformation. Except for the nicotinamide moiety, NADP can be described as binding at the protein surface. In contrast, FAD binds at the surface of the FAD-domain but at the boundary to the NADP-domain, so that only the adenine moiety extends to the protein surface. Oxidised glutathione (GSSG) binds between subunits. There is a close contact between the flavin ring of one subunit and the interface-domain of the other.

As binding of the substrate NADPH to the crystalline enzyme has not yet been observed, we have to discuss its position on the basis of results obtained for NADP-binding. In Fig. 4 the nicotinamide moiety of NADP does not touch FAD. However, in the difference Fourier map the nicotinamide ring and its ribose have a much smaller density than the rest of NADP, indicating that this part is only loosely bound. Therefore, it is likely that the nicotinamide does not assume its correct position, reminiscent of similar experiences in binding studies of NAD to crystalline liver alcohol dehydrogenase^{21,22}. If one keeps the pyrophosphate moiety of NADP in its place, some rotations allow the nicotinamide to take up two positions where it makes contacts with the isoalloxazine ring. In one of these positions nicotinamide would be fixed between two large side

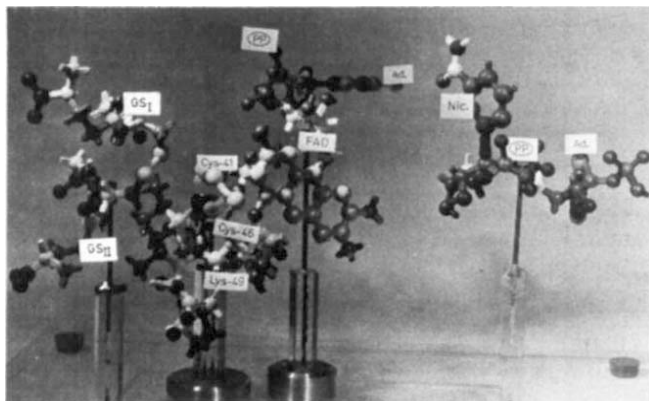
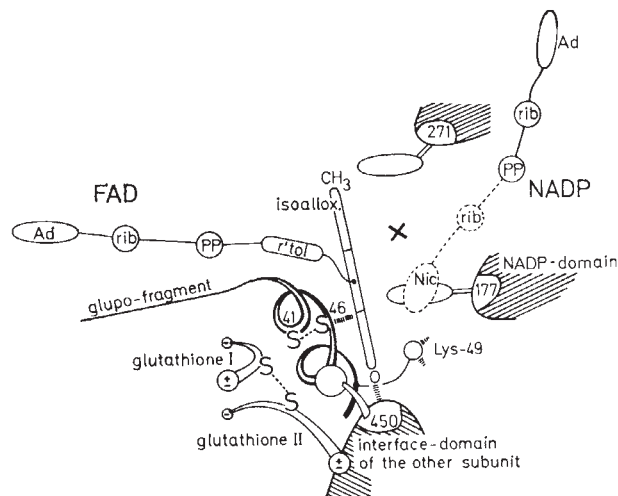


Fig. 4 FAD, NADP, oxidised glutathione (GS_I and GS_{II}) as bound to the crystalline protein. Part of the polypeptide chain around the active disulphide (Cys-41 and Cys-46) is inserted. It is possible to swing the nicotinamide ring round to touch the flavin ring.

chains and would touch the benzene moiety of the isoalloxazine near to C9. The alternative position has been chosen in Fig. 5. Here, nicotinamide touches the pteridinedione moiety of FAD at N1. Lys-49 curls around the flavin (Fig. 4); its amino group touches O4a and presumably forms an internal salt-bridge to residues 181 and/or 353.

The enzyme is stable in its reduced state, so that the catalysis can be divided into two steps, enzyme reduction and subsequent reduction of GSSG^{1,23}. During enzyme reduction, the formation of a transient adduct between C4a of FAD and the proximal sulphur has been postulated³⁰. Moreover, spectroscopic data

Fig. 5 Sketch of the active centre of glutathione reductase. Flavin is hydrogen-bonded through O2a and/or N3 to the backbone of the other interface-domain at residue 450. The glupo-fragment containing the active disulphide is given. The proximal Cys-46 is adjacent to the 4a-carbon of flavin. The side chain of Lys-49 curls around the isoalloxazine and presumably forms a salt-bridge to residues 181 and/or 353. Two large side chains of the NADP-domain are inserted. Nicotinamide may bind at position (X) where it would stack between these side chains. Inserted is the alternative binding position where the nicotinamide ring stacks on one of the large side chains and touches N1 of FAD. The observed densities prohibit stacking of the nicotinamide ring with the isoalloxazine and a contact between nicotinamide and N5 of FAD. GS_I and GS_{II} are stretched between subunits. The large side chain of residue 450 from the other subunit protrudes into the space enclosed by the isoalloxazine, the active disulphide of the protein, and the disulphide bridge of GSSG. This side chain is presumed to be a His that takes part in catalysis.



show that the stable reduced enzyme EH_2 contains a charge transfer complex between a thiolate ion and reoxidised isalloxazine^{24,25}. Our static picture of the oxidised enzyme supports this hypothesis; the proximal Cys-46 is a good candidate for the thiolate ion, and the internal probably charged Lys-49 may enhance the acceptor capacity of the isalloxazine. NADPH may induce the formation of this structure by delivering two reduction equivalents which are transferred by means of isalloxazine to the active disulphide.

For the second part of the catalytic cycle it has been proposed that a base is involved³⁴ and that a mixed protein-glutathione disulphide is formed⁴. According to our geometric interpretation (Fig. 4), the mixed disulphide would include GS_1 because its sulphur is at contact distance to the distal sulphur (of Cys-41). The postulated base may be the side chain of residue 450 of the other subunit, which is surrounded by the cystines of both GSSG and glutathione fragment as well as by the pteridinedione moiety of FAD. According to its size and shape it may well be a His (ref. 26). This interpretation implies that an essential residue of the catalytic site is contributed by the other subunit and that the monomer is inactive.

The following mechanism of action²⁸ is then possible for the second part of the catalysis: 'His'-450 is positively charged (stabilising the proximal S^- and consequently the charge transfer complex) and attracts electrons from sulphur II in GSSG (Figs 4 and 5). This facilitates a nucleophilic attack of the distal sulphur (Cys-41) on sulphur I of GSSG. In a concerted action 'His'-450 is deprotonated, one molecule of GSH (II) is released and a mixed disulphide is formed. In the absence of a positive charge at 'His'-450, the charge transfer complex becomes unstable, the thiolate ion (Cys-46) is now available for a nucleophilic attack on the mixed disulphide and the GS^- (I) is released (compare with ref. 4). General stereochemical features of this thiol-plus-substrate activation by His in enzyme catalysis have already been indicated³³. In order to consolidate our conclusions, we shall refine the geometrical structure and determine the complete amino acid sequence¹⁴.

This work has been accepted by the University of Heidelberg as part of the Ph.D. thesis of E.F.P. We thank R. Thieme for help in preparing figures and the Deutsche Forschungsgemeinschaft for support.

Received 30 December 1977; accepted 3 March 1978.

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The covalent and tertiary structure of bovine liver rhodanese

Jan H. Ploegman, Gé Drent, Kor H. Kalk & Wim G. J. Hol

Laboratorium voor Struhtuurchemie, Rijksuniversiteit Groningen, Nijenborgh 16, 9747 AG, Groningen, The Netherlands

Robert L. Heinrikson, Philip Keim, Litai Weng & John Russell

Department of Biochemistry, The University of Chicago, 920 E. 58th Street, Chicago, Illinois 60637

Bovine liver rhodanese is a single polypeptide of 293 amino acids in which the halves of the molecule assume analogous tertiary structures in the absence of substantial sequence homology. The sulphur atom transferred during catalysis is bound in persulphide linkage to Cys-247. Substrate binding seems to involve Arg-186 and Lys-249.

RHODANESE (thiosulphate:cyanide sulphurtransferase, EC 2.8.1.1) is a mitochondrial enzyme that is widely distributed in nature and especially abundant in mammalian liver and kidney. Its physiological significance in sulphur metabolism has yet to be established firmly. Several recent review articles^{1–4} have dealt with the rhodanese from bovine liver, and this enzyme has been characterised in greatest detail in terms of both its structure and its mechanism of action. Rhodanese reacts with sulphane sulphur-containing anions (for example, thiosulphate) to yield a sulphur-substituted form of the

enzyme in which the sulphur atom is covalently attached to a single cysteine residue. The first product (for example, sulphite) is then discharged. In the second step of this double-displacement mechanism⁵, the enzyme-bound sulphur is transferred to a thiophilic acceptor, such as cyanide, to form thiocyanate and regenerate the free enzyme. Certain features of the active site, in addition to the catalytically functional sulphhydryl group^{6–8}, have been inferred by chemical modification and kinetic studies. These include a cationic substrate binding site⁹, a hydrophobic region^{10,11}, and the possible participation of a divalent metal ion^{12,13}.

The size and number of polypeptides in rhodanese have been subjects of controversy. A molecular weight of 37,500 was initially reported for the enzyme, based on sedimentation-velocity and diffusion measurements¹⁴. Subsequently, numerous lines of evidence suggested that rhodanese was a dimer composed of identical subunits half this size^{8,11,13,15–18}. The dimer theory, however, was later found to be incompatible with the results of molecular weight analyses from several labora-

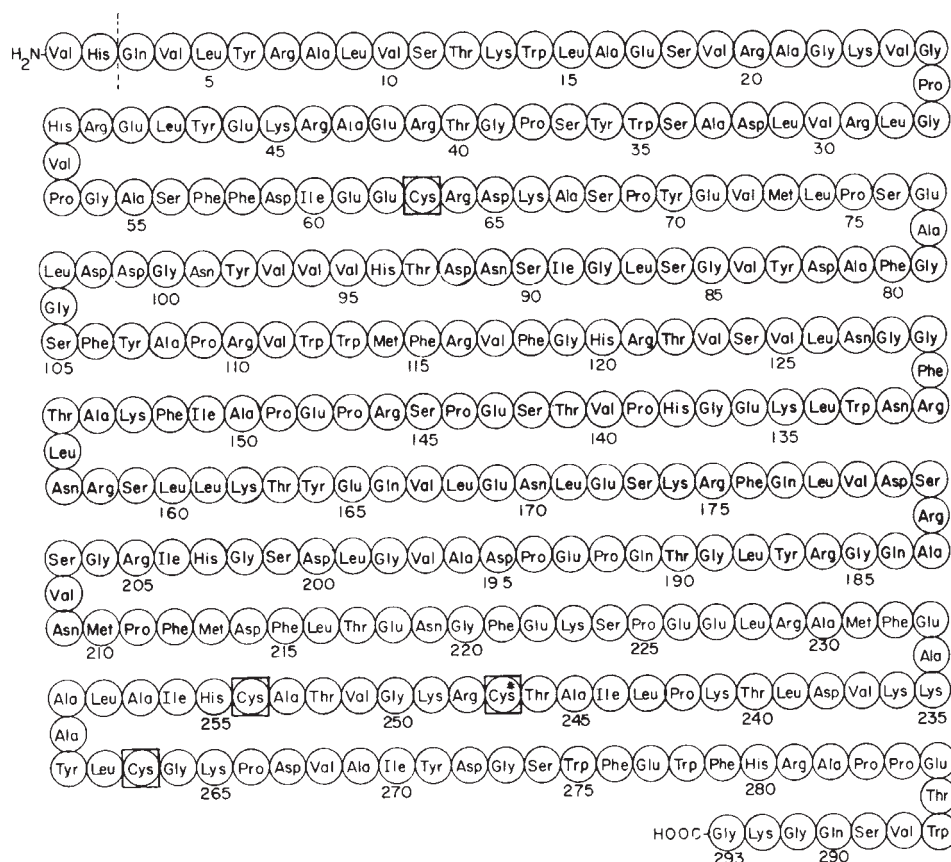


Fig. 1 The covalent structure of bovine liver rhodanese. The positions of the four cysteine residues are indicated by boxes; the active site cysteine 247 is starred.

ories^{15,19-21} and with the preliminary findings derived from structural studies of the enzyme^{22,23}.

We report here covalent and three-dimensional structural studies which are final proof that rhodanese is a single polypeptide chain of 293 residues. The structural analysis of rhodanese is exceptional in that the later stages of the sequence and X-ray studies were carried out in close collaboration, and the exchange of information derived by the two independent approaches proved to be extremely beneficial. The chemical structure is of obvious importance to the interpretation of electron density maps, but the crystallographic study aided the development of strategies for the sequence analysis of certain portions of the molecule that were particularly difficult to solve. Also included here are the results of chemical modification studies formulated to probe the active site of rhodanese.

Primary and tertiary structure determination

Rhodanese was purified from homogenates of bovine liver by fractional pH and ammonium sulphate precipitation²⁴ and ion-exchange chromatography¹⁵. The strategy for the covalent structural determination was based on both automated and conventional Edman degradative techniques²⁵. The majority of the sequence was elucidated by automated Edman degradation of large fragments produced by limited, specific cleavage of *S*-carboxymethylcysteinyl rhodanese. Accordingly, cyanogen bromide fragments were of great importance to the sequence study, as were the large peptides generated by trypsin digestion of preparations in which the lysyl residues were blocked reversibly by citraconylation. The covalent structural determination was completed by conventional analysis of smaller peptides.

Crystallographic analysis²⁶ was carried out on crystals of sulphur rhodanese prepared from a solution at pH 7.3 containing 2.0 M ammonium sulphate and 1 mM thiosulphate. Thiosulphate was removed before data collection by soaking the crystals in an identical solution lacking this substrate.

Several heavy atom derivatives were prepared by soaking crystals in solutions of the appropriate compounds. Those found to be most useful were *p*-chloromercury (II) benzene sulphonic acid, NaReO₄, and NaAu(CN)₂.

X-ray intensity data for sulphur-rhodanese, collected to 2.5 Å resolution, were obtained with a computer-controlled four-circle diffractometer. Isomorphous replacement and anomalous-scattering information were used in the refinement and phase calculation procedure. The mean figure of merit²⁷ for 10,124 reflections was 0.762. The electron density distribution, calculated using centroid phases²⁸, was plotted on Mylar sheets. With the aid of an optical comparator²⁹, a molecular model of the enzyme was constructed of Kendrew-Watson components. The electron density map was of very good quality; many backbone carbonyl groups were visible, no discontinuities in the main chain were observed, and dimpling of several aromatic side chains was apparent. The primary structural information derived from the crystallographic study agrees completely with that determined in the sequence analysis.

Crystals of rhodanese were also studied at 2.9 Å resolution after stripping the crystalline enzyme of substrate sulphur by the addition of KCN, by difference Fourier analysis with the figure of merit as weighting factor³⁰.

The rhodanese molecule

The covalent structure of bovine liver rhodanese is presented in Fig. 1. The molecule is a single polypeptide chain of 293 residues and the molecular weight calculated from this structure is about 32,900. One cysteine residue is located in the first half of the molecule at position 63. The three remaining cysteines in rhodanese are proximally located in sequence near the COOH-terminus at positions 247, 254 and 263; there are no disulphide bridges in the molecule. Earlier chemical modification studies implicated Cys-247 as the site of substitution in sulphur-rhodanese from both bovine liver¹⁵ and kidney³¹.

The spatial distribution of the α -carbon atoms in sulphur-



Fig. 2 Stereodiagrams of the polypeptide chain of sulphur-rhodanese. *a*, The double domain structure viewed along the diad axis; *b*, superposition of the domains following rotation and translation with respect to the diad.

rhodanese is shown in Fig. 2*a*. The molecule can be described approximately as an ellipsoid of dimensions $60 \times 50 \times 40$ Å. It consists of two globular domains of nearly equal size and conformation connected by a loop of extended chain. The NH_2 -terminal domain (I) comprises residues 1–142 and the COOH -terminal domain (II) extends over residues 159–293; residues 143–158 are located in the connecting loop. Residues 1–3 and 291–293 are not visible on the map.

Each domain of rhodanese contains a central five-stranded parallel β -sheet (Fig. 3) with a left-handed twist of about 140° when viewed perpendicular to the direction of the strands (see ref. 32 for conventions). Two α -helices are located on one side of the sheet, and three on the other. In total, 37% of the residues occur in α -helices, 15% in parallel β -structures, and 24% in turns. There are no anti-parallel β -strands in this enzyme. In each domain two of the five helices run anti-parallel to and connect the neighbouring strands of the sheet forming two β - α - β units. The helical crossovers in these units, as well as all the other crossover connections in rhodanese, are right-handed^{32–34}.

Comparison of the primary and tertiary structure of the two domains

As indicated in an earlier structural comparison²³, the folding of the two domains in rhodanese is strikingly similar. This

similarity is readily apparent in the hydrogen bonding scheme and in distance maps²⁶, both of which show symmetries between, as well as within, each domain. For a more quantitative comparison, one domain was rotated and translated into the other in such a way as to minimise the sum of the squared distances between all C_α atoms assumed to be equivalent³⁵. The quantitative measure of similarity, the root mean square deviation, is 1.95 Å for 117 of the approximately 140 C_α atoms in each domain. A stereodiagram showing the superposition of the domains is presented in Fig. 2*b*. Within the error inherent in this operation, the superimposable C_α atoms in the two domains are related by a strict twofold symmetry axis. The angle of rotation deviates $\sim 1^\circ$ from 180° , and the translation parallel to the rotation axis is less than 1 Å. This kind of symmetry between domains in the same polypeptide has been observed in several instances^{36–38}, but not to the extent seen in rhodanese.

In view of the great conformational similarity of the two rhodanese domains, comparison of their sequences might be expected to reveal extensive homology; such is not the case. In fact, given the sequence information alone, a computer-assisted search for homology between the halves of the rhodanese molecule based on minimum base changes per codon generated 19 different 'best' alignments (W. M. Fitch, unpublished). However, as these sequences were no more similar to one

another than to randomised sequences from each half of the molecule, no statistically significant degree of homology was revealed. The same conclusions were reached in a search for homology between the domains by analysis of pentadecapeptide segments selected at random from the molecule (W. M. Fitch, unpublished).

Figure 4 shows an alignment of the sequences in the two domains based on the tertiary structural comparison in Fig. 2b. With the introduction of gaps in the sequences corresponding to loops exposed at the surface of one domain which have no counterpart in the other, the question of sequence homology between the halves of the rhodanese molecule may be examined. Of the 117 pairs of residues with superimposable C_α atoms, 15 are identical, 56 require a single base change, 45 require two base changes, and one pair requires three base changes. A value of 1.27 was calculated for the minimum number of nucleotide base changes required per codon. Similar calculations from comparison of unrelated sequences yield values of about 1.5 (ref. 39). Therefore, the minimum base change criterion indicates some evolutionary relationship between the two domains of rhodanese. Nevertheless, the similarity between the domains is expressed to a greater degree by their tertiary structures than by their sequence homology, even when the sequence comparisons are based on the tertiary structure itself.

In view of these findings, it was of interest to compare the sequences of the two rhodanese domains in terms of the patterns of secondary structure predicted for each according to the rules of Chou and Fasman^{10,11}. The two patterns thus obtained are not only very similar to one another, but correspond closely to the actual conformations observed in the two domains. The comparison of predicted and observed α -helices, β -strands, and turns given in Table 1 reveals that 11 of the 12 helices, 8 of the 10 β -strands, and all of the 17 turns can be predicted from the sequence. A single helix and β -strand are overpredicted, as are six additional turns. Overall, the predictive analysis of rhodanese locates regions of secondary structure within the two domains with an accuracy comparable to that achieved for the 29 globular protein structures that serve as the sample set from which the predictive parameters were obtained¹¹.

Rhodanese, therefore, is an example in which two extensive regions of sequence within the same polypeptide chain assume equivalent tertiary structures in the absence of substantial sequence homology. This poses intriguing questions in regard to the function and evolution of the enzyme. It seems plausible that the two domains have arisen through a process of gene duplication and divergence by means of which their sequences have changed considerably but their conformational similarity has

Table 1 Comparison between the secondary structural elements observed by X-ray diffraction and those assigned on the basis of predictive rules

X ray	Predicted	X ray	Predicted
8-10 β_A	5-10 β	160-162 $\beta_{A'}$	160-164 β
11-22 α_A	11-21 α	163-174 $\alpha_{A'}$	165-175 α
25-28T	25-28T		
27-33 β_B	28-33 β	177-181 $\beta_{B'}$	176-180 β
37-40T	37-40T		
42-50 α_B	41-50 α	183-189 $\alpha_{B'}$	187-191 β
		196-199T	193-196T
52-55T	52-55T	204-207T	200-203T
			204-207T
56-58 β_C	56N	208-210 $\beta_{C'}$	210N
59-63 α_C	57-62 α	211-216 $\alpha_{C'}$	211-216 α
65-68T	63-66T	217-220T	217-220T
69-75N	68-71T		
76-87 α_C	75-82 α ; 85-88T	224-235 $\alpha_{C'}$	225-238 α
90-93T	90-93T	238-241T	239-242T
94-98 β_D	94-98 β	242-246 $\beta_{D'}$	243-247 β
101-104T	99-110 2T	247-250T	247-250T
105-108T			
107-119 α_D	111-119 α	251-264 $\alpha_{D'}$	253-260 α
120-121N	119-122T	265-268T	265-268T
122-127 β_E	122-126 β	269-271 $\beta_{E'}$	268-272 β
126-129T	126-129T	271-274T	272-275T
129-137 α_E	130-136 α	274-282 $\alpha_{E'}$	275-282 α
138-156 Loop	139-142T	283(4)-286(7)T	283-286T
	143-146T		
	150-156 α		
157(8)160(1)T	157-160T		

The following symbols are used: α , alpha-helix; β , beta-strand; T, turn; N, no ordered structure.

been preserved. The selective pressure for maintenance of similar folding patterns in the halves of the molecule would have to operate in accordance with some functional requirement. However, the structural equivalence of the two domains is in sharp contrast to their functional non-equivalence as judged by the current understanding of rhodanese as a sulphur transferase¹. In fact, as will be discussed below, the active site comprises asymmetric contributions from the two domains. Regardless of the evolutionary interpretation of the origin of the domains, the relationship between the evolution, structure and function of rhodanese remains a challenging subject for further inquiry.

Rhodanese and the structure of other proteins

The order of parallel β -strands in each rhodanese domain is CBDEA (see Table 1), where the lettering of the strands

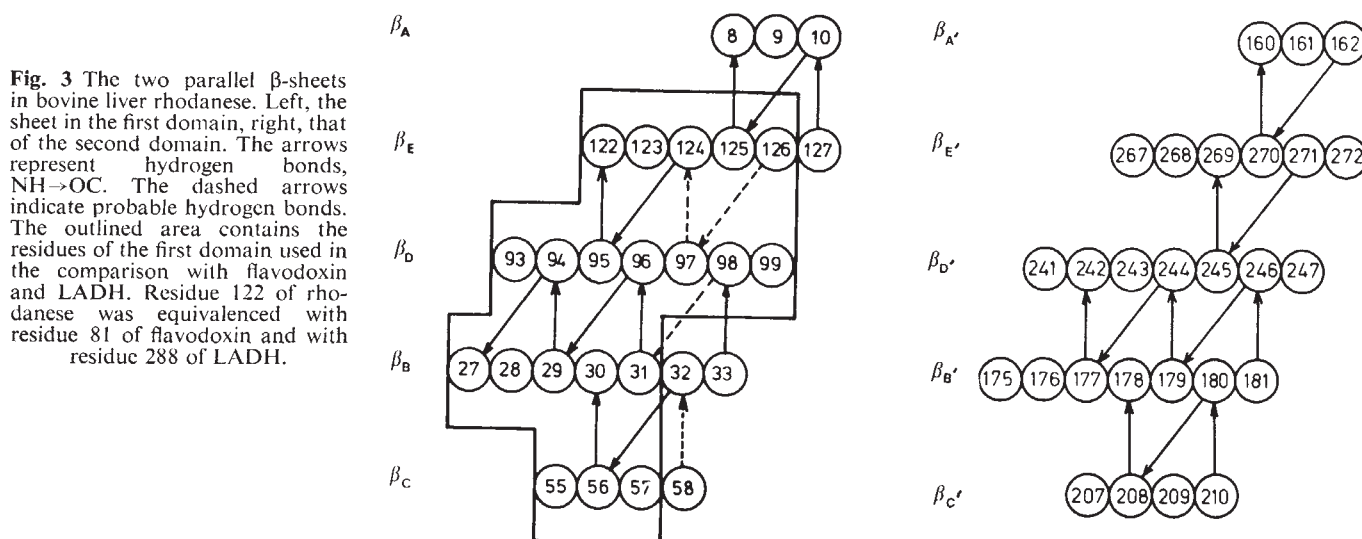


Fig. 3 The two parallel β -sheets in bovine liver rhodanese. Left, the sheet in the first domain, right, that of the second domain. The arrows represent hydrogen bonds, $NH \rightarrow OC$. The dashed arrows indicate probable hydrogen bonds. The outlined area contains the residues of the first domain used in the comparison with flavodoxin and LADH. Residue 122 of rhodanese was equivalenced with residue 81 of flavodoxin and with residue 288 of LADH.

indicates their position in the chain starting at the NH_2 -terminus (Fig. 3). This order is reminiscent of that found in the nucleotide binding domain of the dehydrogenases³⁹ (CBADEF) and in flavodoxin⁴² (BADEF). The topology in all three cases is the same, many β -strands being connected by anti-parallel helices, thus giving rise to β - α - β units. We have performed a quantitative comparison of the structures of rhodanese with both the nucleotide binding domain of liver alcohol dehydrogenase (LADH)⁴³ and flavodoxin⁴². The approximately 20 C_α atoms, assumed on the basis of hydrogen bond patterns and strand order to be equivalent in the β -structures of these three proteins (Fig. 3) were superimposed³⁸ and a comparison of the total domains was made. Stereodiagrams show that the similarity between total domains does not extend far beyond the C_α atoms in the β -structures selected initially for the structural comparisons. This is in marked contrast to the superposition of rhodanese domains I and II (Fig. 2b), in which equivalence is observed for 117 of the C_α pairs. Values of the r.m.s. deviation and minimum base change per codon for the 20 C_α pairs selected in the comparison of rhodanese and LADH are 1.48 Å and 1.55, respectively. The corresponding values for the 19 C_α pairs in rhodanese and flavodoxin are 1.22 Å and 1.21. The structural similarities in these comparisons are restricted to a small proportion of residues comprising parallel β -strands. Therefore, the criteria which can be advanced in support of the hypothesis that the rhodanese domains are the result of divergent evolution from a common ancestor are more compelling than those derived from the structural comparisons of rhodanese with either LADH or flavodoxin.

The active site

The active site is located in a depression between the two domains of rhodanese. The bound substrate sulphur atom is clearly visible in the electron density map of sulphur-rhodanese

and apparently forms a persulphide bond with the S_γ of Cys-247. Cleavage of this persulphide linkage by cyanide is associated with a distinct negative density in the difference Fourier map corresponding to the position originally identified with substrate sulphur. These observations agree with published conclusions regarding the participation of a sulphhydryl group in catalysis (see ref. 2) and the correlation between the carboxymethylation of Cys-247 and loss of enzyme activity⁴⁴. The persulphide accepts hydrogen bonds from the peptide groups of Arg-248, Lys-249, Val-251 and Thr-252 and from the hydroxyl group of Thr-252 (Fig. 5). NH-S hydrogen bonds have been found in several iron-sulphur proteins^{45,46}. The presumed function of the NH-S hydrogen bonds in these proteins is to stabilise negatively charged iron-sulphur complexes, a different number of NH-S bonds possibly giving rise to quite different redox potentials of these complexes. In rhodanese the hydrogen bonds stabilise the persulphide and may, in the sulphur-free enzyme, promote the reactivity of Cys-247. These observations collectively support the view that the stability and reactivity of functional sulphur atoms in proteins may be influenced by a network of hydrogen bonds.

Evidence for the participation of a divalent metal ion in the catalytic mechanism^{2,4,12,13} has been difficult to substantiate⁴. No metal was observed at the active site in the crystallographic analysis and it seems clear from these studies that rhodanese is not a metalloenzyme.

The essential Cys-247 is located at the bottom of a pocket in a turn between a β -strand (β_D) and a helix (α_D). Side chains from both domains I and II form the walls of the pocket and these in turn are largely segregated into hydrophobic (Phe-212, Phe-106, Tyr-107, Trp-35, and Val-251) and hydrophilic (Asp-180, Ser-181, Arg-182, Arg-186, Glu-193, Arg-248, Lys-249, and Thr-252) regions. These findings, therefore, confirm earlier predictions that the active site of the enzyme is made up of cationic⁹ and hydrophobic^{10,11} components. There exists in rhodanese an

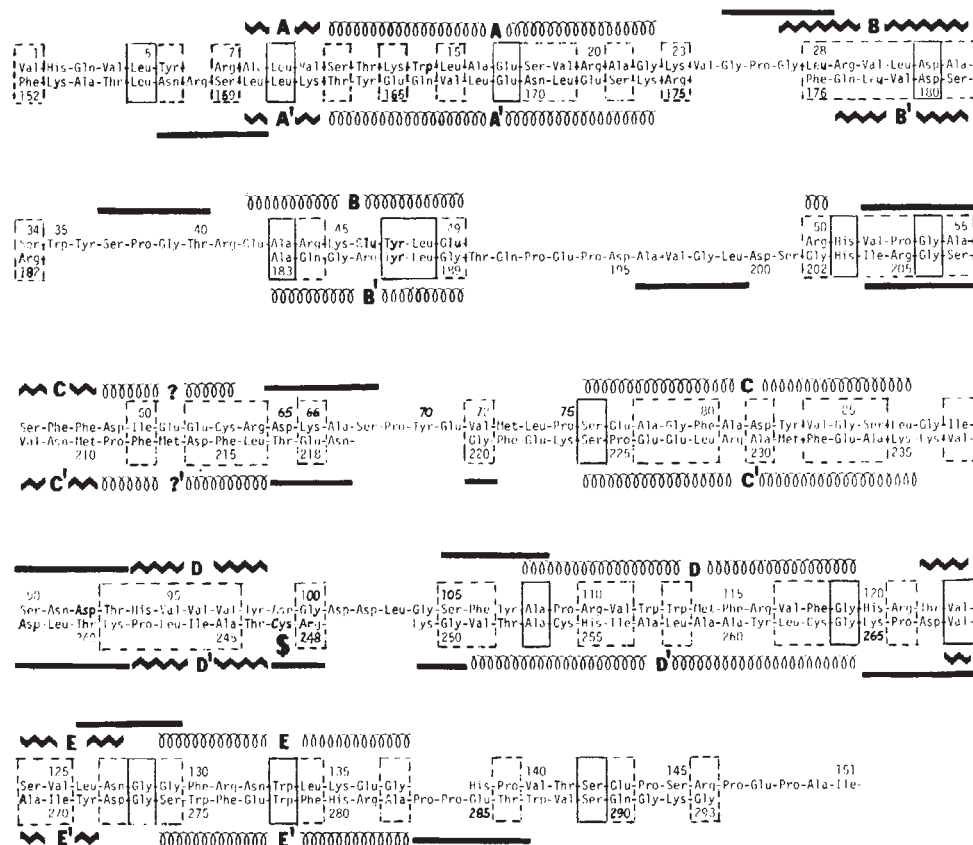
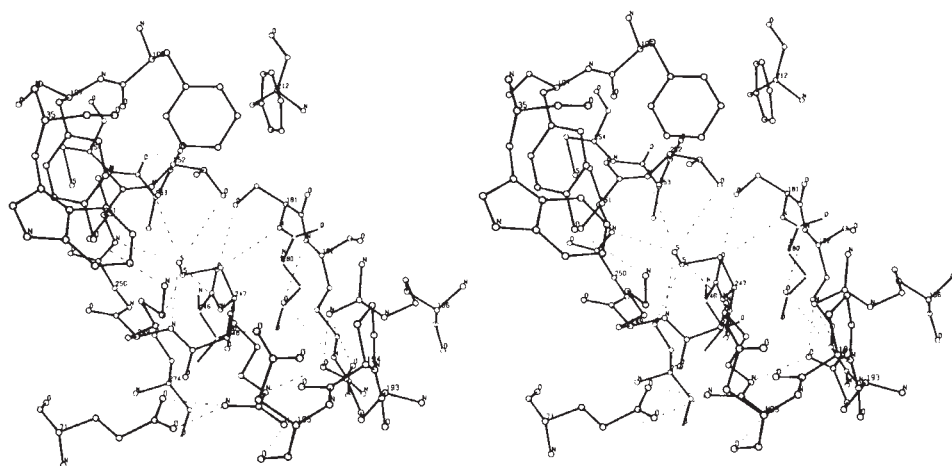


Fig. 4 Alignment of the amino acid sequences of the two rhodanese domains based on the structural comparison in Fig. 2b. Helical (~~~~~) and β (~~~~~) structures are lettered sequentially A-E and A'-E' in domains I and II, respectively. Turns are denoted by solid bars. Identical residues are enclosed in solid boxes, and dashed boxes indicate the residues the codons of which differ by a single base change. The \$ designates the substrate sulphur bound in persulphide linkage to Cys-247. The alignments of residues 1-6 with 152-158 and 143-146 with 290-293 are not based on the tertiary structural comparison.

Fig. 5 A stereodiagram of the active site of sulphur-rhodanese.



Active site of Rhodanese

extensive salt bridge network, and all of the six residues involved are in the vicinity of the active site (Fig. 5).

The crystallographic analysis of rhodanese has identified side chains of potential importance in the catalytic mechanism. The positive charge of Arg-186 and possibly that of Lys-249 are close enough to Cys-247 to participate in binding thiosulphate in the correct orientation. These charges might even polarise the highly polarisable S-S bond in the substrate molecule, thus rendering the outer sulphur atom more susceptible to thiophilic attack by Cys-247. Chemical modification of amino groups has been shown to lead to inactivation of sulphur-rhodanese⁴⁷, although no identification of a particular reactive lysine was made. Reaction of sulphur-rhodanese with a 60-fold molar excess of phenylglyoxal is accompanied by loss of enzyme activity, and the major site of chemical modification has been shown to be Arg-186 (ref. 44). Kinetic analysis shows that the predominant effect of this modification is to increase the value of the apparent K_m . Therefore, these findings, and those of the X-ray analysis, demonstrate the importance of Arg-186 in the sulphur-transfer function of rhodanese.

It is of interest in this regard that if rhodanese is stripped of substrate by reaction with cyanide before the addition of phenylglyoxal, stoichiometric quantities of reagent lead to rapid inactivation without any modification of arginine⁴⁴. Instead, the loss of enzyme activity is accompanied by the formation of disulphide bridges between the essential Cys-247 and either Cys-254 or Cys-263, in the molar ratio of 7:3, respectively. The significance of the phenylglyoxal-mediated synthesis of these disulphide bonds is not clear. Crystallographic studies show that the average positions of the three cysteine side chains do not favour disulphide bond formation; the sulphur atom of Cys-247 in crystalline sulphur-rhodanese is separated from the S_γ atom of Cys-254 and Cys-263 by 7 and 19 Å, respectively. If the polypeptide backbone were sufficiently flexible, then intramolecular chain diffusion could promote disulphide bond formation in the presence of phenylglyoxal. Although calorimetric⁴⁸ and spectral⁴⁹ measurements of soluble rhodanese reflect considerable structural flexibility associated with sulphur binding, difference Fourier analysis indicates that only minor conformational changes occur in the active site region when the bound sulphur is cleaved by treatment of sulphur-rhodanese crystals with cyanide²⁶. We anticipate that knowledge of the primary and tertiary structures of rhodanese will facilitate the reconciliation of these results and will also serve as a basis for further inquiry regarding structure-function relationships in the enzyme.

J.H.P. cannot agree with an evolutionary explanation of the results obtained, as he does not accept the theory of evolution on religious grounds²⁶.

We thank Drs J. Westley and J. Drenth for discussions. The crystallographic part of these studies have been carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.), with financial aid from the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.). The chemical analysis was supported by a grant to R.L.H. (BMS-75-23506) from the NSF. P.K. acknowledges support from the American Heart Association for his established investigatorship.

Received 16 December 1977; accepted 24 February 1978.

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letters to nature

Selection effects in redshift distribution of QSOs

Two selection effects have been applied^{1,2} to the distribution of emission line redshifts of QSOs. However, since this hypothesis was proposed, emission-line redshift values have almost trebled. I report here a new analysis of 639 redshifts to discover whether they exhibit these selection effects for various values of peak width.

One of these selection effects is due to the finding that more QSOs have been observed at redshift intervals where there are more search lines available for redshift measurement. Significant correlation was obtained between the quantity A_s , which is a measure of the 'availability of search lines', and the distribution of redshifts comprising 202 objects. The second effect is due to the fact that the emission lines of QSOs on entering the U, B, V filters may change the colours of the objects such that the colour indices have values similar to those of main sequence stars, some QSOs being thereby lost due to misidentification. Significant correlation was again obtained, for $z > 0.7$, between the quantity $(U-B)_L$, which is a measure of the probability of missing some QSOs due to the change in the colour index $(U-B)$, and the redshift distribution comprising 265 objects.

As the emission line redshift values have almost trebled since then, I have reinvestigated the above effects using updated data. Furthermore, some doubts have been expressed by Karlsson³ about these effects. However, his comments are based on rather qualitative work without computing any mathematical correlation between the relevant quantities. Furthermore, his analysis is restricted to the 'broad peaks' in the distribution, comprising bins of 0.1 in z ($\Delta z = 0.1$), whereas most of the redshift distribution analyses in literature have been done with narrower peaks. Finally, the catalogue of Burbidge *et al.*⁴ combined with the list of Osmer and Smith⁵ has provided us with 639 QSOs which is a somewhat larger sample than that used by Karlsson. We were, therefore, prompted to make a thorough analysis of this sample of 639 redshifts to find out whether they exhibit the two above mentioned selection effects, for various values of peak width (Δz).

Evaluations of A_s and $(U-B)_L$ have been extended up to $z = 3.6$. As in the previous analyses A_s has been computed for $\Delta z = 0.01$, $\Delta z = 0.05$ and $\Delta z = 0.1$ and $(U-B)_L$

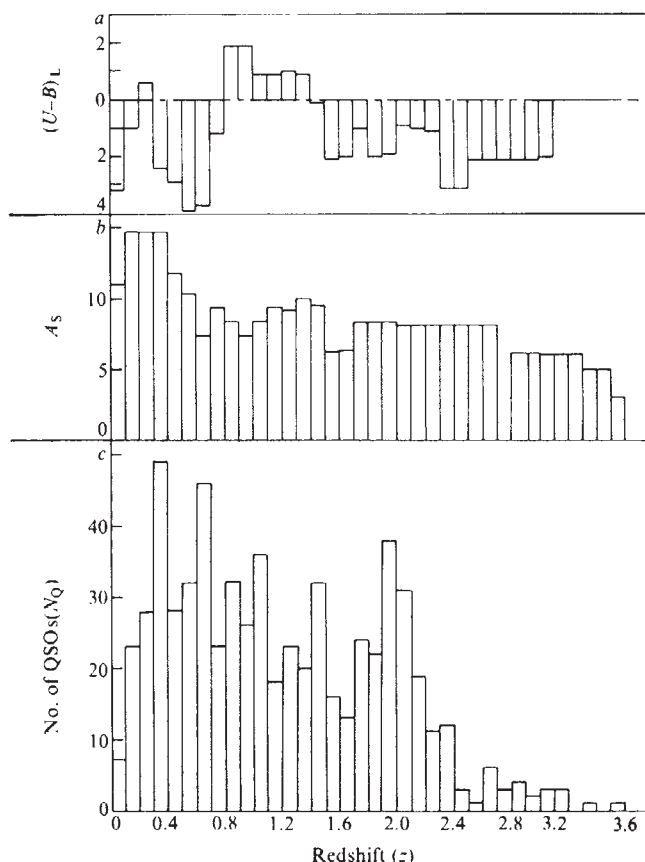


Fig. 1 Distributions of $(U-B)_L$ (a) and A_s (b) and the emission line redshift distribution for the present sample of QSOs (c). $(U-B)_L$ is a measure of the probability of missing some QSOs due to change in the colour index $(U-B)$ by the appearance of emission lines in U and B bands. A_s is a measure of the availability of search lines.

for $\Delta z = 0.05$ and $\Delta z = 0.1$. Figure 1 shows the distributions of the quantities $(U-B)_L$ and A_s and also the emission line redshift distribution for the present sample of QSOs, for $\Delta z = 0.1$. The correlation coefficients are presented in Table 1 for the runs of redshift distribution (N_Q) against each of the distributions of A_s and $(U-B)_L$ over relevant ranges of redshift and for various values of Δz .

Table 1 Correlation coefficients (r) and percentage significant levels (L) for the runs of redshift distribution (N_Q) against each of the distributions of A_s and $(U-B)_L$

N_Q/A_s						$N_Q/(U-B)_L$							
Redshift range 0.0-3.6						Redshift range 0.0-3.6				Redshift range 0.7-3.6			
$\Delta z = 0.01$		$\Delta z = 0.05$		$\Delta z = 0.1$		$\Delta z = 0.05$		$\Delta z = 0.1$		$\Delta z = 0.05$		$\Delta z = 0.1$	
r	L	r	L	r	L	r	L	r	L	r	L	r	L
0.5840	<0.1	0.5795	<0.1	0.4263	<0.1	0.0981	40	0.0340	85	0.3493	1.0	0.3699	3.8

For A_s against N_Q all the correlations are statistically significant and for $(U-B)_L$ against N_Q the correlation is significant only for $z > 0.7$. This confirms that the results of the previous analyses^{1,2} hold good with updated data for all the different values of the peak width (Δz). It is concluded that the two selection effects under consideration are present in the redshift distribution of the present sample of QSOs and should be borne in mind for any physical interpretation of the distribution.

D. BASU

Department of Physics,
University of the West Indies,
Kingston 7, Jamaica

Received 3 January; accepted 28 February 1978.

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Solar wind effect on Jupiter's non-*Io*-related radio emission

JUPITER'S decametric radio emission has been monitored for over 20 years since its discovery in 1955¹. In 1964 Bigg² discovered that the decametric radiation is strongly modulated by the first Galilean satellite, *Io*. It has subsequently been established³ that there are other types of decametric radio activities which are not modulated by *Io*. We show here that these non-*Io*-related (NIR) decametric radio activities are affected by solar wind conditions around Jupiter.

The existence of solar wind modulation in the NIR activities has been a matter of controversy³. Kovalenko⁴ has shown that NIR activity occurs preferentially about 10 d after the large enhancement of geomagnetic activity. Using superposed epoch analysis, Kennedy *et al.*⁵ demonstrated a possible correlation between NIR activity and the solar wind sector structure. Recently Oya and Morioka⁶ have found a positive correlation between NIR activity and the geomagnetic activity index Kp in a dynamic cross correlation analysis. From power spectrum analysis, however, Kaiser and Alexander⁷ concluded that there was no significant peak around the 25-d period; such a peak would be expected if the solar-wind effect were to exist. It should be noted that in many previous studies NIR activity has been compared with the solar wind properties linearly extrapolated from observations near 1 AU. It is now known that the development of the solar wind structure during its expansion towards the outer heliosphere cannot be treated linearly and the effect of the stream-stream interaction must be incorporated⁸. The

forward and reverse shock pairs are found to be the dominant structure near Jupiter's orbit⁹. Since the dynamic pressure varies over nearly two orders of magnitude in association with the passage of the stream interaction region at the orbit of Jupiter⁸, it is of great interest to investigate the effect of the passage of the stream interaction region on the structure of Jupiter's magnetosphere.

We shall compare NIR activity with the solar wind conditions at Jupiter inferred from the solar wind parameters (velocity V_{sw} , density N , and pressure P) observed at 1 AU, by using the nonlinear one-dimensional (radial) hydrodynamic equation system⁸.

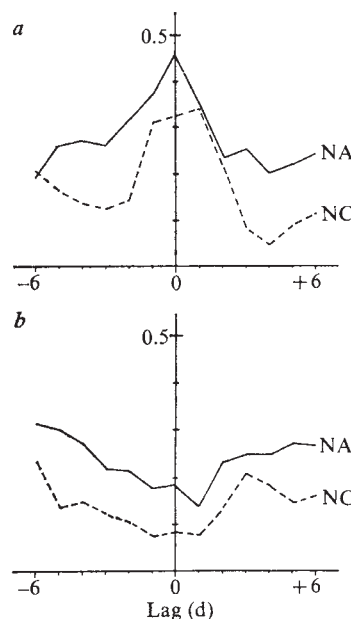


Fig. 2 The occurrence probabilities of the non-*Io*-related A (main) source and C (late) source on each day between -6 days (advanced) and $+6$ days (delayed) from the arrival of the solar wind with high dynamic pressure (*a*; $NV_{sw}^2 > 1.6 \times 10^5$), and with low dynamic pressure (*b*; $NV_{sw}^2 < 2 \times 10^4$).

For our study we have selected the data of 1974 apparition for two reasons. First, a clear 27-d recurrence pattern of the solar wind structure observed at 1 AU during this year¹⁰ justifies our assumption that the effect of the longitudinal separation between the Earth and Jupiter can be incorporated by simply adding a corotational delay (or advance) to our one-dimensional (radial) solution. Second, we can test the accuracy of the calculation by comparing the calculated solar wind profile in the distant heliosphere with the observations performed by Pioneer 10 (5.4–6.5 AU) and Pioneer 11 (41–5.0 AU).

Comparison of the calculated position of the stream interaction regions with the findings of Pioneers 10 and 11 (refs 11, 12) shows that our calculation of the solar wind evolution can reproduce the shock position within ± 1 d error in the arrival time.

In Fig. 1, the calculated solar wind profiles at Jupiter between days 180 and 355 of 1974 are shown. Solar wind parameters followed are, velocity V_{sw} , number density N and dynamic pressure NV_{sw}^2 . In Fig 1c, showing NV_{sw}^2 the shaded, large enhancements of NV_{sw}^2 (nearly two orders of magnitude) represent the passage of the interaction region at Jupiter. The NIR (non-*Io*-related A and C) activities are selected from the catalogue of observation at the University of Colorado¹³, using the criteria for the source classification described by Carr and Desch³. The reported intensities of NIR activities in three quantisation levels are plotted in Fig. 1d.

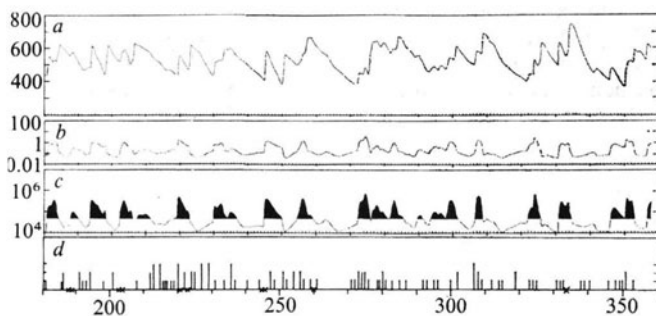


Fig. 1 The calculated time profiles of the solar wind parameters: *a*, V_{sw} km s⁻¹; *b*, N cm⁻³; *c*, NV_{sw}^2 cm⁻³ km² s⁻², and *d*, the variation of the intensity of Jupiter's non-*Io*-related decametric radiation. The date of the opposition in this apparition is September 5, or DOY (days of year) 248 in 1974.

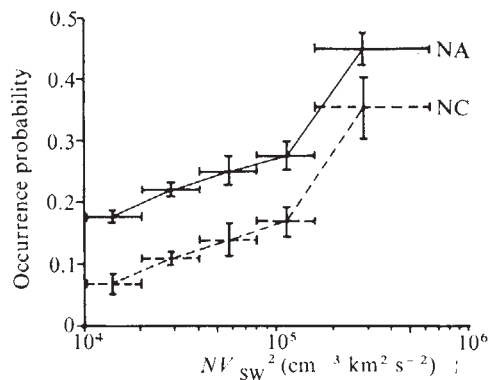


Fig. 3 Correlations between NIR occurrence probabilities and dynamic pressure NV_{sw}^2 . 1974 Apparition, DOY 180–355. NA, non-Io-related A source; NC, non-Io-related C source.

In the correlation analysis between NIR activity and the solar wind, we have used the daily average values of the calculated solar wind parameters, as decameter observations were performed once daily.

Among the parameters tested, the best correlation was found between NIR and the dynamic pressure NV_{sw}^2 . The result of the superposed epoch analysis is shown in Fig. 2. In Fig. 2a the occurrence probability of NIR activity defined by the ratio [total time when NIR activity is observed (min)] ÷ [total observation time (min)] in each day of the period between –6 days (advanced) and +6 days (delayed) from the arrival of the solar wind stream with $NV_{sw}^2 > 1.6 \times 10^5 \text{ cm}^{-3} \text{ km}^2 \text{ s}^{-2}$ is shown for non-Io related A source (NA) and non-Io-related C source (NC) separately. It is seen that the NIR sources become most active when the solar wind with large NV_{sw}^2 arrives at Jupiter. The inverse relationship is seen between NIR activities and the solar wind with low NV_{sw}^2 in Fig. 2b, where the occurrence probability reaches the minimum up to 1 d after the arrival of the solar wind with $NV_{sw}^2 < 2 \times 10^4 \text{ cm}^{-3} \text{ km}^2 \text{ s}^{-2}$.

The effect of NV_{sw}^2 on NIR activity is further confirmed in Fig. 3, where occurrence probabilities of NA and NC are calculated for five bins of NV_{sw}^2 for lag = 0 day. (The size of bins is chosen in such a way that all bins have almost the same number of data points). It is readily seen that there is a positive correlation between NIR activity and NV_{sw}^2 . Since the typical error of the total time of events/observations in each bin in Fig. 3 is several tens of minutes, which is less than ~ 2% of the total time of observations, the main contribution to the error in the estimation of the occurrence probability would come from the inaccuracy in the calculation of solar-wind evolution. To obtain some indication of the reliability of these correlations, we have calculated variations of the occurrence probabilities corresponding to ± 0.5 d error in the estimation of the solar-wind arrival time. These variations are shown as vertical bars in Fig. 3.

We have shown that Jupiter's non-Io-related radiation shows a good correlation with the dynamic pressure of the solar wind. The high dynamic pressure region in the solar wind would collide with and activate Jupiter's magnetosphere, and enhance the non-Io-related radiation.

It should be noted that there is an alternative interpretation of the results: in the interaction region the interplanetary magnetic field **B** is compressed^{9,14} so the interplanetary electric field — $V_{sw} \times B$ seen in the rest frame of Jupiter becomes large. If this electric field can penetrate into Jupiter's magnetosphere probably through the magnetic field reconnection, processes analogous to the Earth's substorm activity might result. From this analysis we cannot yet distinguish which of these is the most likely process of the activation of Jupiter's magnetosphere.

We thank Drs A. Nishida, H. Washimi and O. Saka and Professor T. Obayashi for useful discussions. The data on

Jupiter's radiation are provided by Dr J. W. Warwick and his colleagues. Solar wind data are from WDC-A rockets and satellites.

T. TERASAWA

K. MAEZAWA

S. MACHIDA

*Institute of Space and Aeronautical Science,
University of Tokyo, Komaba, Meguro-ku,
Tokyo 153, Japan*

Received 21 November 1977; accepted 7 March 1978.

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Has the Sun really got a companion star?

HARRISON¹ has pointed out that the six radio pulsars with the smallest observed spin-down rates ($\dot{P}$) are grouped together in a relatively small region of the sky, roughly in the direction of the galactic centre. To explain this particular effect he suggested that the intrinsic values of $\dot{P}$ are actually larger than the observed ones, because the barycentre of the Solar System would be accelerated ($\ddot{r} \approx 10^{-8} \text{ cm s}^{-2}$) in that direction. Harrison then suggested that this acceleration would be due to an hitherto undetected companion star of the Sun. He proposed a faint white, red (or even black) dwarf in a closed orbit, rather than a gas-accreting neutron star or black hole in an open orbit. We show here that a companion producing such an acceleration should almost certainly have been discovered because of its brightness in the infrared or visible region of the spectrum, depending on the character of the object. We conclude that Harrison's explanation is probably incorrect, and therefore we presume that the observed spin-down rates of these six pulsars (including the binary pulsar) are intrinsic and that their extreme values are due to the particular way in which the pulsars were formed. We show that the apparent clustering of these radio pulsars is a consequence of a strong observational selection effect and conclude that the distribution of all radio pulsars seems to be symmetric around the galactic centre.

In general, a companion star of the Sun will perturb the motions of the planets. Its presence will cause an additional perihelion advance of the planetary orbits. One can show that, at present, only the orbit of the planet Mars will be perturbed by a measurable amount by the action of the companion star. From the uncertainty in the observed and calculated planetary orbit² we can derive a lower limit of $0.3 M_{\odot}$ for the companion's mass which is incompatible with the allowed upper limit in the luminosity (as will be shown later). An analysis on the longitudinal residuals of Neptune to delimit the possible space for a perturbing body, has been carried out by Rawlins and Hamerton³. Their solution implies an extra acceleration of $2 \times 10^{-9} \text{ cm s}^{-2}$, which is, in our context, a negative result. Both calculations, however, are made under the assumption that the companion moves in a circular orbit. Generally, this will not be the case and, unfortunately, for noncircular orbits a

Table 1 Effective temperature, apparent visual magnitude, infrared K magnitude, period, distance, proper motion and annual parallax for a selection of masses, all producing an acceleration of $10^{-6} \text{ cm s}^{-2}$ in our (known) Solar System.

Mass ($M_{\odot}$)	Emission source	T_{eff} (K)	V magnitude ($\lambda = 0.55 \mu\text{m}$)	K magnitude ($\lambda = 2.2 \mu\text{m}$)	Period (yr)	Distance (AU)	Proper motion (arc s yr $^{-1}$)	Annual parallax (arc s)
0.01	Albedo = 0.44	—	9.0	—	670	80	1,900	2,700
0.015	Albedo = 0.44	—	10.2	—4.0	900	95	1,400	2,200
	Black dwarf	680	24.7					
0.02	Albedo = 0.44	—	11.0	—5.3	1,150	110	1,100	1,900
	Black dwarf	820	18.0					
0.08	M dwarf	2,600	—3.3	—	3,200	220	400	950
0.1	M dwarf	2,700	—3.9	—	3,800	240	350	850
1	White dwarf	8,000	—4.3*	—	21,000	770	60	250

*For a white dwarf the V magnitude is independent of its mass (see text).

mass limit cannot be derived, but nor are the effects on the orbit of Neptune available. Therefore, we consider all possible kinds of companion stars, including massive planets and compact objects.

Suppose that the companion is a (degenerate) black dwarf with a low mass (0.01 – $0.07 M_{\odot}$), which has the age of the Solar System ($t \approx 4.5 \times 10^9$ yr). The luminosity of such an object, which has failed to ignite hydrogen, can be derived from the model calculations by Graboske, as has been done by Tarter⁴:

$$L = 1.7 \times 10^6 M^{2.2} t^{-0.837}$$

(luminosity L and mass M in solar units; age t in yr). The mass–radius relation as a function of age for these models is⁴

$$R = 0.29 M^{-0.22} t^{-0.096}$$

(R in solar radii). Both relations are valid in the mass range 0.015 – $0.020 M_{\odot}$ (the luminosity agrees well with the one derived by Staller⁵). These equations yield a mass–temperature relation which determines the energy distribution, assuming that the black dwarf radiates as a black body. The distance d to the companion is known from the required acceleration:

$$\dot{v} = GM/d^2 = 10^{-6} \text{ cm s}^{-2},$$

or $d \approx 800 M^{1/2} \text{ AU}$ (M in solar units).

The apparent magnitudes in the near infrared K -band ($\lambda = 2.2 \mu\text{m}$) for these low mass objects are given in Table 1. For convenience, the period, distance, proper motion and annual parallax are also tabulated. Table 1 shows that in the $2\text{-}\mu\text{m sky}^{6,7}$ a black dwarf of $0.015 M_{\odot}$ should be comparable with Betelgeuse, the brightest infrared object north of declination $\delta = -33^\circ$. More massive black dwarfs are even brighter. Less massive dwarfs are closer, will seriously disturb the orbits of the planets, and moreover, will brightly reflect the light of the Sun, like a planet. A black dwarf of $0.01 M_{\odot}$ (distance 80 AU) with a jovian albedo* will have an apparent visual magnitude due to reflection of $m_v = 9.0$ and would, therefore, have been included in the HD catalogue. In view of its large proper motion, it seems highly unlikely that such an object would never have been recognised.

Stars more massive than $0.08 M_{\odot}$ with the age of the Solar System are dwarfs of spectral type M or earlier, situated on the main sequence. Calibrated absolute magnitudes of red dwarfs were taken from Vecder⁹. Their expected apparent visual magnitudes (see Table 1) leave no doubt that the companion cannot be a red dwarf.

A white dwarf, 4.5×10^9 yr old, is also excluded. Its apparent visual magnitude should be $m_v = -4.3$, independent of its mass. (This is due to the particular form of the mass–flux and mass–distance relations.) Absolute magnitudes were calculated from the cooling curves as given, for example, in ref. 10.

The remaining possibilities are a neutron star or a black hole. These objects will accrete matter from the interstellar (or interplanetary) medium with a capture rate¹¹ of

$$\dot{M} \approx 4\pi G^2 M^2 \rho / v^3$$

where G is the gravitational constant, ρ is the density (typically $10^{-24} \text{ g cm}^{-3}$), and v is the greatest value of the sound speed and the velocity relative to the gas far from the compact object; v is not expected to exceed 100 km s^{-1} (bound or open orbit). For $M \approx 0.5 M_{\odot}$ (a reasonable lower limit) we obtain a value of at least $\dot{M} = 6 \times 10^7 \text{ g s}^{-1}$.

A neutron star will convert this matter partially into radiation with an efficiency factor ζ , producing a luminosity $L = \zeta GMM/R$ ($R \approx 10 \text{ km}$ is the radius of a neutron star). If this energy is emitted from the neutron star surface in the form of black body radiation (a typical temperature is 10^5 K) we expect, assuming $\zeta \approx 0.1$, a visual magnitude of $m_v = 6.8$ for a velocity of 100 km s^{-1} . Lower velocities will give rise to a still brighter source, the brightness being nearly independent of the mass. In which energy range the radiation will escape is, however, not certain. If the infalling protons thermalise just at the surface of the compact object, the radiation will be in the form of γ rays with a typical energy of 10 MeV . The details of the thermalisation process, which are difficult to estimate, will ultimately determine the energy spectrum. Therefore, although it seems very unlikely, the existence of such a nearby neutron star cannot yet be fully excluded. Similar remarks can be made for an accreting black hole. Estimates^{12,13} for the intensity and spectrum of the emerging radiation depend on the assumed dissipation processes for the inflowing particles before they are definitely captured by the hole.

Taking into account the extremely low probability for a very close encounter with just such a compact object (a closed orbit is not acceptable according to the present theories of binary evolution¹), we conclude that little room is left for an undetected solar companion star that produces an acceleration of $10^{-6} \text{ cm s}^{-2}$. Therefore, we will from here on assume that the extremely low period derivatives of the six radio pulsars are intrinsic.

A direct observational consequence of this assumption is that we expect a strong concentration towards the galactic plane as well as a symmetric distribution around the galactic centre. Indeed all these six pulsars are found within $|b^{\text{II}}| < 7^\circ$; in contrast, their occurrence in galactic longitude is limited to $0^\circ < l^{\text{II}} < 90^\circ$ (note that this region is intersected by the ecliptic; this formed the basis of Harrison's suggestion for a solar companion). Table 2, however, strongly supports the supposition that this is due to a bias in the observations: in the region ($|b^{\text{II}}| < 7^\circ$, $-90^\circ < l^{\text{II}} < 0^\circ$; only observable from Earth south of $\delta = -33^\circ$) $\dot{P}$ has been determined for only three pulsars. The lack of an extreme case among these three is not surprising in view of the ratio of the pulsars with an exceptional

Table 2 The 94 pulsars known within ($|b^{\text{II}}| < 7^\circ$, $|l^{\text{II}}| < 90^\circ$)¹⁴

	$90^\circ > l^{\text{II}} > 0^\circ$	$0^\circ > l^{\text{II}} > -90^\circ$
Total no. known	74	20
$\dot{P}$ determined	35	3
Extreme value ¹ for $\dot{P}$	6	0

$\dot{P}$ to those with a 'normal' $\dot{P}$ as observed between $l^{\text{II}} = 0^\circ$ and 90° (see Table 2). Therefore, we conclude that the distribution of all pulsars is most probably symmetric around the galactic centre. Of course, confirmation must come from more $\dot{P}$ measurements.

Thus, we feel that Harrison's suggestion for a solar companion is premature and the reason that these six radio pulsars are exceptional remains to be resolved.

We thank E. P. J. van den Heuvel and R. J. Takens for stimulating discussions. The work was supported by the Netherlands Organization for the Advancement of Pure Research, Z.W.O.

Note added in proof: Wright's discussion¹⁵ concerning a supermassive black hole as a solar companion is restricted to the case of a circular orbit for this companion.

H. F. HENRICHS
R. F. A. STALLER

Astronomical Institute,
University of Amsterdam,
Roetersstraat 15,
NL-1018 WB Amsterdam, The Netherlands

Received 6 February; accepted 7 March 1978.

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Rotation period of comet Donati

COMET Donati, 1858VI, was a spectacular comet, famous for its coma with multiple haloes, seen, described, and measured by many observers using optical telescopes. The haloes were roughly parabolic envelopes with vertices towards the Sun forward from the tail axis and the 'foci' near the apparent nucleus of the comet. The envelopes appeared to be uniformly spaced radially from the nucleus. Because of the regularity and observed sharpness of these haloes, I assume here that they represent repetitive ejection of material from a single active area exposed successively to solar radiation as the solid cometary nucleus rotated¹.

Schmidt² at Vienna, in observations between 3 and 18 October 1858, measured the angular radii of the outer edges of these haloes, in a direction perpendicular to the apparent tail axis and through the nucleus as centre—the 'semi-latus rectum', p , of each parabolic envelope. Only the inner one or two haloes were sharp enough for reliable measurements. Each measured p divided by the angular velocity of ejection, then represents the time interval, Δt , since the beginning of each halo ejection. The instant of observation minus Δt gives a zero-date, (ZD) for each halo.

For the linear expansion velocity of such haloes, v , seen perpendicular to the line of sight, I use Bobrovnikov's³ results, represented by the equation:

$$v = 0.535r^{-0.6} \text{ km s}^{-1} \quad (1)$$

where r is the solar distance measured in AU.

With equation 1, r and geocentric distance, Δ , a simple calculation reduces the measured p to Δt and thence to ZD . Each 'observation' of p by Schmidt combines some 10 micrometer measures. I have taken the mean of his observations for each halo and the corresponding mean time. Table 1 lists the results of these calculations, using all Schmidt's p observations.

As an example of the primary calculation, consider Table 1, revolution number 21 on 7 October. Schmidt identified it as halo h , with a mean $p = 34.05''$. At $r = 0.602$ AU, equation 1 gives $v = 0.975 \text{ km s}^{-1}$. With $\Delta = 0.556$ AU, the expansion velocity is $155.4'' \text{ d}^{-1}$ and $\Delta t = 0.219$ d. Struve and Winnecke⁴ earlier observed the same halo from Pulkova. The ZD calculated from their combined measures is 0.009 d later than Schmidt's.

To check the validity of equation (1), the solution for the period includes a linear correction factor to $1/v$, with the result, including σ 's:

$$ZD = 0.19253 \text{ dn} + 3.4862 \text{ d} - \text{Observation date} - 1.071\Delta t \pm 0.00017 \pm 0.050 \quad (2)$$

The check on equation 1 is reassuring, because the correction factor to Δt or $1/v$ is only $7 \pm 5\%$. The σ in residuals for ZD s involving five or more observations is 0.014 d. The period is $4.621 \text{ h} \pm 0.004 \text{ h}$.

Table 1 Data of halo initiation comet Donati, 1858 VI

Rot. no. (n)*	Obs. no.†	Δt (d)	Obs.‡ 1858	O-C§ (0.001d)
0	2	0.244	3.748	0
1	2	0.063	3.746	0
5	5	0.251	4.740	+22
6	11	0.090	4.746	+8
10	4	0.286	5.729	+11
11	10	0.109	5.723	+2
16	15	0.183	6.774	+11
20	3	0.352	7.727	+14
21	20	0.219	7.742	-22
25	1	0.336	8.711	+52
26	13	0.240	8.728	-21
31	7	0.284	9.750	-9
32	7	0.102	9.760	+3
37	1	0.170	10.775	-17
41	3	0.323	11.746	+20
42	11	0.187	11.750	-22
57	17	0.257	14.721	-14
62	18	0.272	15.710	-5
67	15	0.293	16.710	+11
73	5	0.136	17.704	+17
78	3	0.162	18.704	+27

*Arbitrary no. of rotation.

†No. of Schmidt's observations involved.

‡Observed date in UT October 1858.

§Residuals in the ZD s observed minus the computed, from a least-squares solution for the period.

I would be interested in an explanation for such regularity in comet activity not involving the rotation of a discrete nucleus. The true period of rotation must, of course, differ from the derived value, which is a synodic period with respect to the Sun for a comet in a highly eccentric orbit. The correction from synodic to sidereal period is trivial, ~ 0.0002 d, its sign depending upon the unknown sense of rotation. More importantly, the active area undoubtedly migrates somewhat over the surface of the nucleus, suggested by an increase in period during the time interval of Table 1. The order of magnitude of the migration rate is not yet well established. The formal standard deviation in the period greatly overestimates the real accuracy.

Having developed the ZD -method for use on another comet, I was surprised to find that Schmidt⁵ had already applied the concept. He obtained a period of 4.27 h for comet Donati, making use of his own expansion velocity measures. Had Schmidt extended his analysis a bit further, the delay in the basic understanding of comets might have been reduced by a number of decades.

The method of ZDs as applied to P/Schwassmann-Wachmann I 192511 leads to a period of 5.0 d (ref. 6). The vector of axial rotation also has been found. From studies of the rotation periods of a few other comets, I find that the 5.0 d period seems to represent an extreme in the distribution function, which, with logarithmic argument in the period, peaks in the general neighbourhood of 0.5 d. Perhaps the extreme of 5.0 d is simply chance. P/Schwassmann-Wachmann I, however, appears to have much the largest nucleus of any regularly observed comet, with radius ~ 10 km or more. Can its slow rotation period be genetic? Did larger comets acquire less rotational velocity than smaller comets during their agglomeration?

A study of comet Donati is underway, utilising a mass of observational data which may provide information about the polar vector. These two comets are rare in that the activity, at least during certain intervals, is confined largely to single areas. Presumably the remaining areas of the nuclei are covered by an insulating crust of meteoritic debris preventing solar radiation from sublimating the underlying ices.

The short period of 4.6 h for comet Donati places it near the danger point of equatorial ejection by centrifugal force. The critical period is near 3 h for icy spheres. In fact a small secondary nucleus was observed for a day or so in comet Donati. If the comet is a homogeneous sphere its density must exceed 0.5 g cm^{-3} . Such a short period for one comet supports the concept that spin-up may indeed be the cause of otherwise unexplained comet splitting. With certain assumptions regarding the time lags in sublimation, the period of a rapidly spinning rough surfaced icy nucleus can be shown to decrease by asymmetrical jet action.

I thank Brian G. Marsden for a computer printout of orbital data for comet Donati. This research was supported by grant NSG 7082 from the US NASA.

FRED L. WHIPPLE

Harvard-Smithsonian Centre for Astrophysics,
Cambridge, Massachusetts 02138

Received 6 January; accepted 28 February 1978.

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Tetrahedral hydration of ions in solution

THE structure of electrolyte solutions is not yet known unequivocally despite its significance both in understanding the structure of liquids and in such areas as ion transport in membranes where both the number of water molecules and their disposition about an ion are of crucial importance. Using a new difference Raman spectroscopic technique, which makes it possible to suppress a large fraction of the spectrum of solutions, we have detected Raman bands which arise from vibrations of waters of hydration in the first hydration shell about alkali ions. The depolarisation and frequency data indicate that the oxygen atoms are tetrahedrally arranged about lithium and sodium ions.

The nature of solvation and structure in these solutions is not completely unknown; a variety of physical methods has given some partial answers. For example, X-ray diffraction gives an indication of the most probable interatomic distances. However, the geometry which is deduced from these experiments must be inferred from the number of water molecules surrounding the ions and the assumption that the geometrical arrangement of these molecules is determined by coordination number in the same way that the number of substituents determines the geometry of ordinary molecules or polyatomic ions. Similar ambiguities arise when other methods are used. We wish to observe an experimental quantity which is directly related to molecular structure. Raman spectroscopy can potentially provide such data.

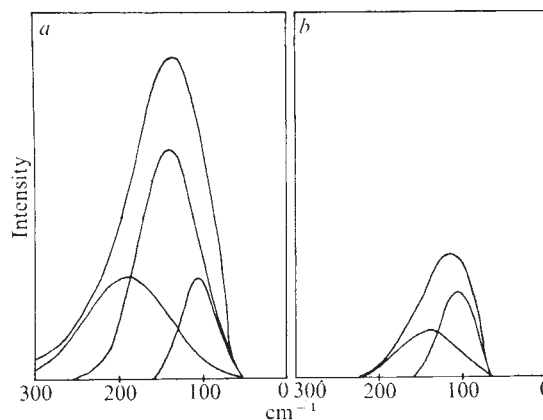


Fig. 1 Difference Raman spectra for a 2.64 M LiCl solution against water: *a*, polarised spectrum; *b*, depolarised spectrum. The Rayleigh intensity arising from the Cl^- ion has been subtracted. The gaussian bands were calculated by curve fitting techniques.

Our double-beam Raman difference method is described elsewhere¹. In this investigation we recorded difference Raman spectra between aqueous alkali halide solutions and pure water. The intensity differentials observed in the low-frequency region result from new bands in the solution spectra which are not present in the Raman spectrum of water, from shifts in the bands belonging to liquid water induced by the ions, and from the removal of bands belonging to the solvent. Hence both positive and negative differences may arise. Some of the new bands are a result of ion solvation in the solutions. By comparing difference spectra obtained for various salts, we have been able to determine which new bands arise from cation-water interactions and which are due to anion-water interactions.

Typical results for an aqueous LiCl solution compared with water are shown in Fig. 1. Also shown are the components of the low-frequency bands. We used digital and analogue curve fitting procedures to decompose the spectra. Two symmetric gaussian bands were used to fit the depolarised spectrum. Their positions and widths were maintained unchanged in fitting the polarised spectrum. Using this strategy we found that a third band was needed to fit the polarised spectrum, with the third band falling at 190 cm^{-1} . Thus, the 190 cm^{-1} band is completely polarised. The random motions which occur in solutions tend to depolarise the light scattered by these solutions, whereas only a highly-symmetric, well-defined geometry can give rise to a completely polarised Raman band. In particular, the scattering species must belong to a cubic (tetrahedral or octahedral) point group. We suggest that the 190 cm^{-1} band is the totally-symmetric vibration (A_1) of the four water molecules against the central ion in a tetrahedral $\text{Li}(\text{OH}_2)_4^+$ complex comprising the ion and its first hydration shell. This geometry has already been proposed on the basis of *ab initio* calculations². Furthermore, a coordination number of four has been indicated for the hydrated Li^+ ion by neutron and X-ray diffraction measurements^{3,4} and ion activity functions⁵. The Raman spectrum therefore indicates with little doubt that there are four water molecules about Li^+ , with their oxygens tetrahedrally disposed.

The origins of the 105 cm^{-1} and the 140 cm^{-1} features will be discussed elsewhere. Broadly, they arise from interactions with the anion and from perturbations in the spectrum of water. In addition to the feature at 190 cm^{-1} , another band at approximately 400 cm^{-1} has also been assigned to $\text{Li}(\text{OH}_2)_4^+$. This band is readily seen in Fig. 2 growing in with increasing LiCl concentration above other bands arising from water libration. Band resolution with a curve resolver indicates that the 400 cm^{-1} and the 190 cm^{-1} bands grow rapidly with increasing Li^+ concentration. The 400 cm^{-1} band is depolarised.

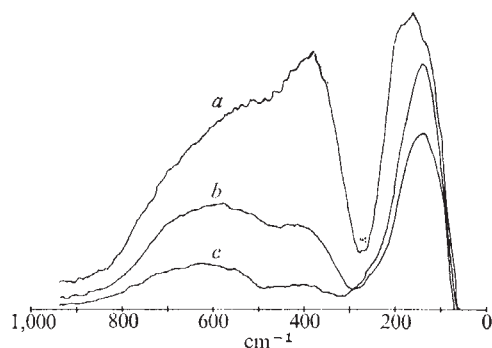


Fig. 2 Concentration dependence of polarised difference Raman spectra for LiCl solutions against water. *a*, Saturated solution; *b*, 7.48 M; *c*, 2.64 M.

We assign this band to the asymmetric vibration (T_2) of the water molecules about Li^+ .

The force constants which would give rise to the assigned LiO frequencies can be estimated by taking $\text{Li}(\text{OH}_2)_4^+$ to be an XY_4 tetrahedral molecule in which Y is taken for simplicity to be a single particle of mass 18 AMU. If we further assume that one can neglect interactions between Li-O stretching and bending motions, one can write for the frequencies of the A_1 and T_2 vibrations the approximate formulae: $\lambda_{A_1} = \mu_y(f_r + 3f_{rr})$ and $\lambda_{T_2} = (\mu_y + 4/3\mu_x)(f_r - f_{rr})$. λ is related to the frequency ν by $\lambda = 0.59(\nu/1000)^2$ (ref. 6). μ_y and μ_x are 1/18 and 1/7 respectively (the inverse masses of water and lithium); f_r and f_{rr} are respectively the Li-O stretching and Li-O, Li-O interaction force constants. With these formulae we obtain $f_r = 0.38$ and $f_{rr} \approx 0 \text{ m dyn } \text{\AA}^{-1}$. The value of f_r so obtained may be compared with the force constant of $0.59 \text{ m dyn } \text{\AA}^{-1}$ reported by Clementi and coworkers⁷ for the monohydrated lithium ion $\text{Li}(\text{OH}_2)^+$ as calculated by an *ab initio* SCF molecular orbital technique, and the $0.6 \text{ m dyn } \text{\AA}^{-1}$ value reported by Rao *et al.*⁸ for the LiO force constant in Li salts of certain organic compounds. The lower value undoubtedly arises from the fact that more than one water molecule is bonded to the ion in $\text{Li}(\text{OH}_2)_4^+$, and may therefore suggest a certain measure of covalent bonding between the lithium and oxygen atoms.

One should note in passing that the frequency of the totally symmetric vibration (190 cm^{-1}) is much lower than the frequency reported by Clementi for $\text{Li}(\text{OH}_2)^+$ (444 cm^{-1}). This arises only partly from the lower force constant, since a diatomic molecule with atoms of mass 7 and 18 AMU and a force constant of $0.38 \text{ m dyn } \text{\AA}^{-1}$ has a harmonic frequency of 360 cm^{-1} . More importantly, in the tetrahedral complex the lithium atom does not move in this vibration and only the reduced mass of water enters into the corresponding G matrix element⁶, whereas in $\text{Li}(\text{OH}_2)^+$ the reduced mass is dominated by the lighter atom and the result is a higher stretching frequency. This same reason accounts for the fact that the infrared spectra of hydrated lithium salts show features almost exclusively at around 400 cm^{-1} . In the infrared the asymmetric stretches in which the lithium accounts for the major part of the motion are expected to give rise to the strongest bands.

As a further check on our conclusions regarding the solvation of the lithium ion, we recorded difference spectra for aqueous LiBr solutions against water. The results which we obtained were nearly the same as those which we have described for LiCl except that the polarised band in the LiBr spectrum occurs at somewhat lower frequency than its LiCl counterpart, presumably because of anion perturbation.

The difference spectra obtained when D_2O is the solvent are very similar to the corresponding H_2O spectra. In particular, the spectrum for LiCl in D_2O shows a polarised band near 190 cm^{-1} which is part of a broad, asymmetric envelope. The

component band positions for the D_2O solution are shifted no more than a few cm^{-1} with respect to their H_2O counterparts, consistent with the above assertion that only the mass of the water appears in the G matrix for the 190 cm^{-1} vibration. The change in reduced mass from 1/18 for H_2O to 1/20 for D_2O will produce only a 5% shift in the frequency of the A_1 vibration. These data further support our conclusion that the 190 cm^{-1} band arises from a tetrahedral structure.

We have also examined the spectra of solutions of sodium salts. NaCl and NaBr both yield difference spectra having three overlapping gaussian bands. The two lower-frequency bands have frequencies similar to those for LiCl and LiBr; the highest-frequency band for NaCl and NaBr is at 170 cm^{-1} and is completely polarised. Using the data of Clementi *et al.*⁷ for $\text{Na}(\text{OH}_2)^+$, we calculate a frequency of 172 cm^{-1} for the totally symmetric stretch of the tetrahedral complex, in excellent agreement with what we observe. This suggests that aqueous Na^+ is also found in a tetrahedral $\text{Na}(\text{OH}_2)_4^+$ structure. This result may have important implications for studies of sodium ion transport in biological systems.

We thank the NRC of Canada, the Atkinson Foundation, and the Research Corporation for support.

K. H. MICHAELIAN

M. MOSKOVITS

Lash Miller Chemical Laboratories and Erindale College,
University of Toronto,
Toronto, Ontario M5S 1A1,
Canada

Received 14 November 1977; accepted 1 March 1978.

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Blocking defects in β'' -alumina observed by high resolution electron microscopy

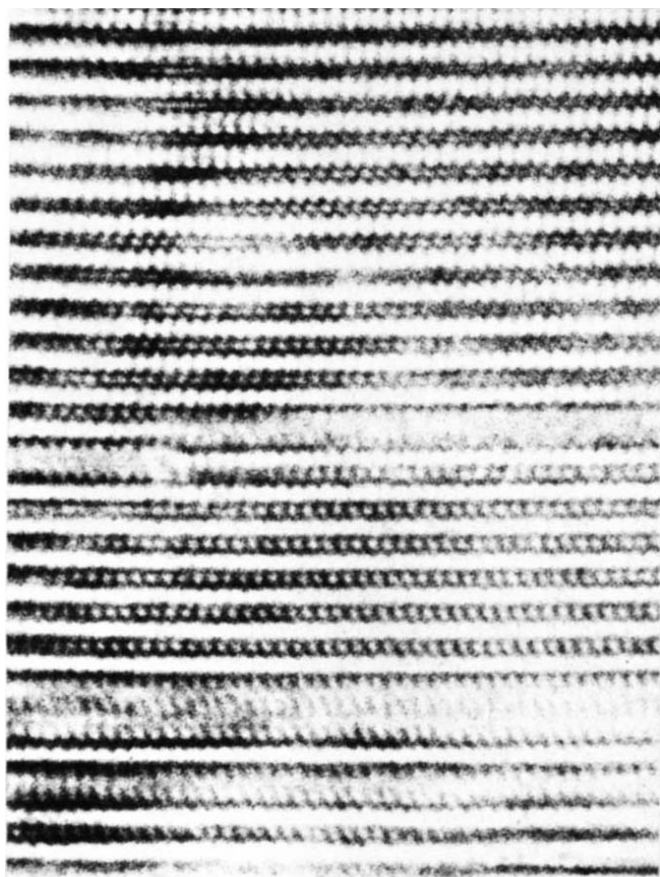
THE sodium-sulphur battery containing the solid electrolyte β'' -alumina is one of the most scientifically intriguing and widely studied battery systems ever discovered. A major reason for this is its potential use in vehicular and energy storage applications. Practical Na-S batteries are constructed with ceramic tubes of Mg-stabilised β'' -alumina as solid electrolyte (U.S.P. 3446677 or 3475225)¹ and a considerable amount of research work on such batteries with β -alumina or β'' -alumina has been done. The greatest area of uncertainty with β'' -alumina is the effect on cell performance of certain changes in the electrolyte with time; serious changes are loss of sodium ions from the positive electrode side of the electrolyte and changes in structural characteristics. We report here, for the first time, an investigation to reveal the defect structure of the crystals of β'' -alumina using high resolution electron microscopy.

Magnesium stabilised β'' -alumina, idealised formula $\text{Na}_2\text{MgAl}_{10}\text{O}_{17}$, has a hexagonal layer structure² with the lattice constants $a = 5.614 \text{ \AA}$ and $c = 33.85 \text{ \AA}$. The structure consists of blocks of four layers of cubic close-packed oxygen ions with appropriate aluminium and magnesium ions in both octahedral and tetrahedral positions. The cubic close-packed blocks are often referred to as spinel blocks. Three such blocks

in β'' -alumina are related to each other by a threefold screw axis and are held apart by planes containing Al–O–Al bridges. All the Na^+ is contained in these planes between the spinel blocks. If the four layers of the cubic close-packed block of oxygen ions are labelled A, B, C, A, and the layer containing the sodium ions is labelled Na; the stacking of layer in β'' -alumina will be $-\text{NaABCANaCABCNaBCABNaABCANa}-$. The distance between the sodium ion layers is $11.28 \text{ \AA}$. Mg stabilised β'' , $\text{Na}_3\text{MgAl}_{10}\text{O}_{17}$, can be compared with the ideal β -alumina, $\text{NaAl}_{11}\text{O}_{17}$. β'' can be obtained by replacing one Al^{3+} by Mg^{2+} and compensating for the lost positive charge by adding another Na^+ ion. The actual composition used in batteries and prepared here is $\text{Na}_{1.6}\text{Mg}_{0.67}\text{Al}_{10.33}\text{O}_{17}$. The Na_2O content in the conducting layers is very similar to β -alumina. It has been proposed³ that the excess of Na^+ ions in the layer of β'' -alumina is compensated by Al^{3+} ion vacancies in the spinel blocks. Another possibility is to compensate for excess of Na^+ in the conducting layers by varying the width of the spinel blocks.

A pressed pellet of a mixture of NaCO_3 , MgO and Al_2O_3 in the mole ratio mentioned above was heated in an electrical furnace at $1,400^\circ\text{C}$ for 5 h in air. The sample was rapidly cooled to room temperature in air. An X-ray powder pattern revealed the same unit cell of β'' -alumina as previously reported². A holey carbon film with the powdered sample was transferred to a Philips EM400 electron microscope, equipped with a high resolution goniometer and operated at 120 kV. To increase resolution⁴, and magnification, the specimen was lifted in the objective lens. Lattice images of thin edges of the crystals were recorded with the a -axis parallel to the electron beam.

Fig. 1 Electron micrograph of a thin crystal of β'' -alumina obtained with the beam parallel to the $[10\bar{1}0]$ direction, showing the defects of spinel blocks of different width and the defect blocking two ion conducting layers of Na^+ . The magnification can be deduced from the periodicity $c/3 = 11.28 \text{ \AA}$, clearly seen between the dark fringes of the spinel blocks. The point-to-point resolution is approximately $3.5 \text{ \AA}$.



All the crystals of β'' -alumina studied so far were rich in defects of the kind shown in Fig. 1. The distance between the dark fringes in the upper part of the electron micrograph is $11.28 \text{ \AA}$ ($= c/3$). The dark fringes represent an image of the projected charge density of the spinel blocks of the structure. The ion conducting layers of Na^+ are represented by the white areas between the dark fringes. This interpretation is verified by the agreement between the observed and calculated images. The latter was computed from the atomic parameters² of β'' -alumina using the multislice method^{5,6}. The centre of Fig. 1 shows the normal spinel blocks of $11.28 \text{ \AA}$ interrupted by a spinel block of $20.3 \text{ \AA}$. This thickness is equivalent to a spinel block seven oxygen layers wide. The defect shows another important feature just to the left of the centre of the figure. A conducting Na^+ layer is blocked by the broad spinel block at the same time as another spinel block of the same width

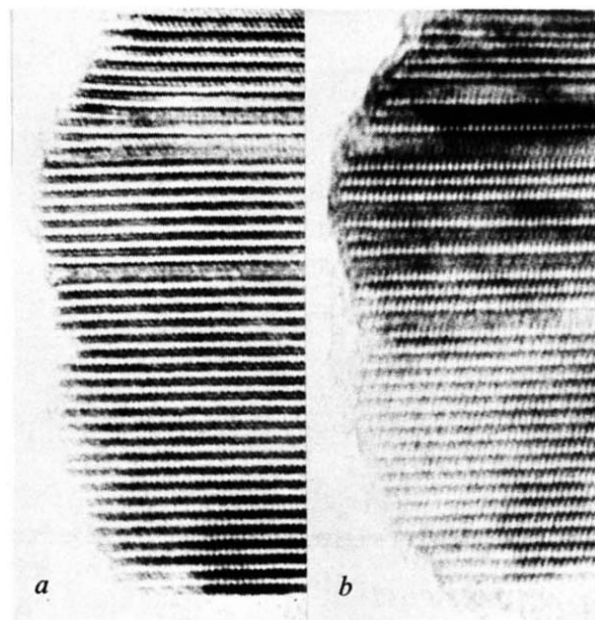


Fig. 2 Two electron micrographs of the same crystal of β'' -alumina obtained with the beam parallel to the $[10\bar{1}0]$ direction showing the effect of the beam irradiation; *a*, at an initial state and *b*, after a few minutes. The magnification can be deduced as in Fig. 1.

blocks the succeeding Na^+ layer. This shows directly for the first time how an ionic conducting layer can be blocked by a defect in the crystal; this phenomenon was frequently found in crystals of β'' -alumina. The lower part of Fig. 1 introduces two wide spinel blocks. The widest one is $29.5 \text{ \AA}$ which is exactly equivalent to an 11-oxygen layered broad spinel block. The other block is equivalent to the one in the middle of the figure. The variation of the width of the spinel blocks must be balanced by a variable Na^+ concentration in the conducting layers.

By careful heating of crystals with the electron beam, the images start to change. The most remarkable feature is that some of the ion conducting layers vanish. Such changes are illustrated in Fig. 2*a* and *b*. The virgin state is shown in Fig. 2*a*, where the normal fringe spacing $11.28 \text{ \AA}$ is interrupted in the upper part of the crystal by three spinel blocks of different width. The two upper defects show a strange behaviour near the crystal edge. We believe that the blocks are splitting up because the conducting layers are losing Na^+ ions and are about to be filled by oxygen ions and Al/Mg ions. Figure 2*b* shows how the wider spinel blocks complete after heating. New wide spinel blocks have also been introduced and one of them has not yet completed near the crystal edge. It is known⁷ from Ag^+ - β -alumina, $\text{AgAl}_{11}\text{O}_{17}$, that the conducting ion can

migrate under the influence of the electron beam and this is obviously what takes place here. The conditions can probably be compared with that of the battery. The Na^+ , in that case, moves out from the conducting layers to react with the liquid sulphur. At the battery's working temperature, a diffusion of Al^{3+} , Mg^{2+} and O^{2-} giving larger spinel blocks competes with an equivalent amount of liquid sodium transformed into Na^+ and moved through the solid electrolyte to fill up the conducting layers continuously. Hence more blocking defects can be produced and the material gradually loses its conductivity; this will cause a decrease in the Na^+ concentration close to the liquid sulphur, which agrees with earlier observations⁸. Introduction of broad spinel blocks as defects is also supported by line broadening of the X-ray powder pattern lines 210 and 217 observed in β -alumina used for a long time in a Na-S-battery.

The investigation of β'' -alumina by high resolution electron microscopy will continue in our laboratory and will include materials used for extended periods in battery systems to reveal more about the defect structure of crystals from cycled materials.

JAN-OLOF BOVIN

*Inorganic Chemistry 2,
Chemical Center,
P.O. Box 740,
S-220 07 Lund,
Sweden*

Received 23 January; accepted 3 February 1978.

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The key to sources of the Pliocene and Pleistocene glaciation is at the bottom of the Barents Sea

DURING the 23rd expedition of RV Academic Kurchatov, 27 cores of bottom sediments were taken from the Rybachiy Peninsula-Franz Josef Land profile¹. The lithological data and the record of pollen analysis revealed the Lower Cretaceous deposits in the basement of two cores in the northern part of the sea. They are overlain by 'ancient clays' (tills)—dark-grey, grey and brown-grey loams and clays of average ($1.6-1.8 \text{ g cm}^{-3}$) and high ($2.0-2.2 \text{ g cm}^{-3}$) density. These clays are shown here to have commenced deposition 3.5 Myr ago, proving that the Barents Ice Sheet (BIS) existed in the late Pliocene. This indicates when glaciation in the mid-latitude began, and why alternation of glacial and interglacial periods occurred.

Ancient clays are fragments of sandstones, limestones, dolomites and sometimes of silicon and coal. In the southern part of the Barents Sea, less than 100 km from the shore, grit and gravel composed of crystalline rocks of Scandinavian origin occur. The ancient clays in the central and northern parts of the sea are characteristically more compact, dark grey in colour, and have a lumpy texture. They contain numerous rounded lumps of dry clay and aleurolite, similar to those of the Lower Cretaceous. The mineral composition of the Lower Cretaceous rocks, lumps and the matrix of ancient clays is very similar. Ancient clays may include redeposited fragments of mollusc shells as well as redeposited Cretaceous and Palaeogenic pollen. Ancient clays must have formed through the exaration of loose Palaeogenic and Cretaceous rocks. The ancient clays

are overlain by the late Glacial varved clays and the Holocene silts, their thickness ranging from 0.2 to 0.5 m (on elevation) to 5.5 m (in the Northcape trough). The ancient clays (mostly in the upper part) are interlayered by varved clays and silts up to 0.7 m thick containing a small amount of arctic and boreal microfauna.

The age of the deposits was determined using the palaeomagnetic method that was successfully used to study morainic deposits². According to evidence from cores 80, 28, 44 and others (Fig. 2), the ancient clays began to be deposited at the end of the Gilbert epoch (about 3.5 Myr ago).

Thus, the existence of the BIS in the late Pliocene has been proved, confirming a supposition first made in 1966^{3,4}. The BIS, therefore, existed when the bed of the Barents Sea was dry land because of lowering in the ocean level. The BIS blocked rivers flowing northwards and made them flow southwards, to the Caspian Sea, which resulted in the Akchagyl and Apsheron transgressions (3.3-0.7 Myr ago). During this period the glaciation of Iceland and Greenland took place^{5,6}. Iceberg deposits were laid down in the Arctic basin, in the Labrador Sea, and on the Rockhall plateau⁷. The latter area could be penetrated by icebergs only under the influence of the BIS. The glaciation of Polar regions in the late Pliocene and the early Pleistocene made the climate of mid-latitudes of Europe and North America become more continental and checked their glaciation.

The rise of the sea level caused the disappearance of the BIS by the end of the Mutuyama epoch. Then conditions similar to the present set in. Slight cooling due to cosmic factors (the Milankovitch curve) might have triggered a chain reaction of ice-sheet growth in Scandinavia and in arctic regions of North America. Glaciation spread over large areas, the areas covered with sea-ice and snow growing concurrently. The Earth's albedo was increasing. Consequently, the warming of the climate due to cosmic factors could not bring about the disappearance of the glaciers. The solar radiation was not absorbed by the Earth as it would be reflected by the vast white surface. Under such conditions, the glaciation of mid-latitudes might have persisted continuously through many millions of years.

Fortunately, the periods of glaciation were alternatively followed by interglacial periods. Cooling was accompanied by the decrease in precipitation. The study of atmospheric circulations reveals that the increase in solar radiation by 6% would have caused a 27% increase in precipitation⁸. In colder periods forests would be substituted by arid periglacial landscapes^{9,10}. The mammoth fauna appeared consisting mainly of huge herbivores^{11,12}. Dry cold periods corresponded to the accumulation of loess deposits¹³. The levels of drainless lakes dropped. The rise in the level of the Caspian Sea was caused by the inflow of glacial waters and not by the increase in the humidity of the climate^{14,15}. Only in the west of North America was cooling accompanied by increase in precipitation and transgressions of lakes.

Decrease in precipitation was responsible for the equilibrium which set in the ice sheets and prevented them from growing. But, for the glaciers to begin their retreat, an additional factor was necessary. This was created by the reappearance of the BIS^{16,17}. The ablation of the present glaciers of the Spitsbergen, the Franz Josef Land, and the northern part of the Novaya Zemlya is less than the accumulation of snow on their surface. This is because during the glaciations the sea level dropped to about 150 m and hence shallow waters around the islands became dry land. In these conditions the formation of icebergs was interrupted, glaciers began to grow, spreading over the adjoining parts of the shelf. According to Weertman¹⁸, the boundary separating the grounding and the floating shelf glaciers runs at a depth of about 200 m. The glaciers could have advanced down to the present 350 m isobath ($150 \text{ m} + 200 \text{ m} = 350 \text{ m}$), thus forming the BIS.

During the maximum of the last glaciation (18,000 yr BP), the BIS reached enormous dimensions (Fig. 1). It spread over the northeastern part of the Kola Peninsula, the whole of the

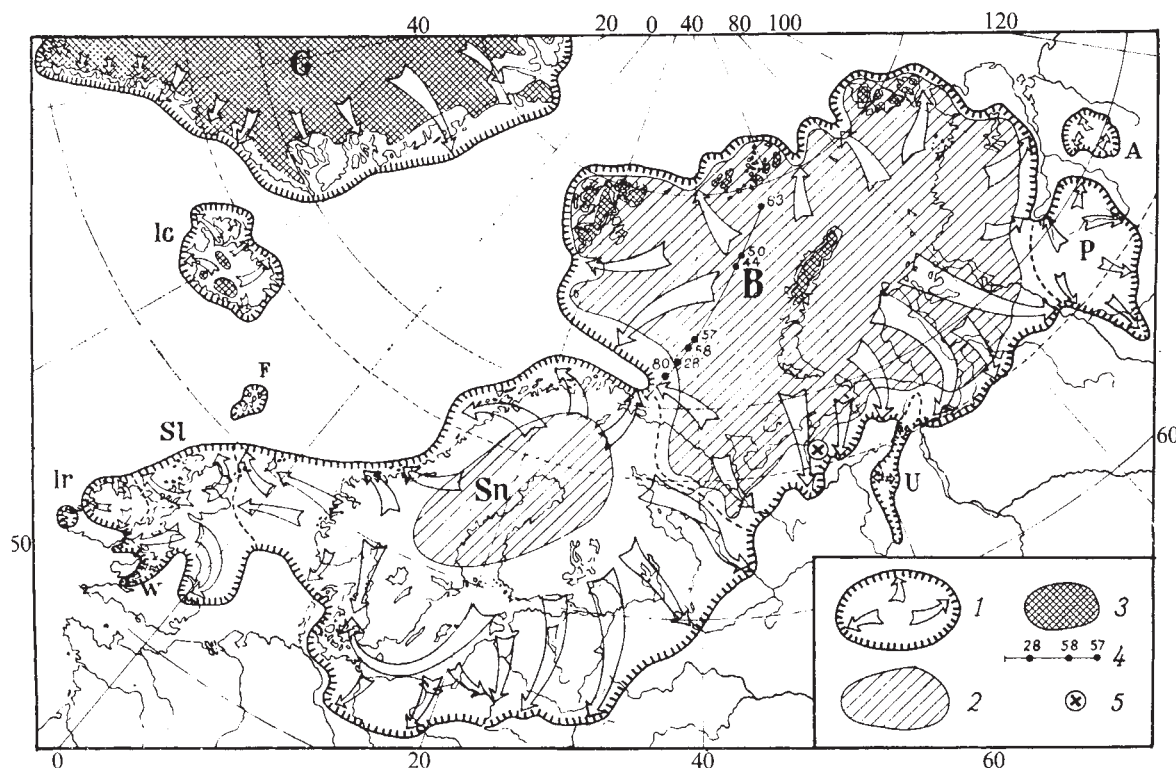


Fig. 1 Glaciation of Northern Europe and adjoining areas. 1, The glaciers 18,000 yr BP; 2, the glaciers 9,500 yr BP; 3, present day glaciers; 4, part of the 23rd expedition of RV Akademik Kurchatov, during which 27 cores of bottom sediments were taken: the numbers correspond to the cores shown in Fig. 2; 5, the region of radiocarbon dating for Barents Ice Sheet advance at 9,500 yr BP. Ice sheets: Sn, Scandinavian; B, Barents (according to the data of M. G. Grosswald, A. S. Lavrov, V. I. Astachov and others); P, Putorana; A, Anabar; Sl, Scotland; Ir, Ireland; F, Faroe; Ic, Iceland; G, Greenland. Mountain glaciers: U, Ural; W, Wales.

Kanin Peninsula, and the northern part of the Pechora basin^{19,20}. It has been discovered recently that the north of Western Siberia was penetrated by the glaciers from the North (the Kara Sea subsidence) rather than from the West (the Urals) or the East (the Putarana Mountains)²¹. On the Kara shelf lay the eastern part of the BIS, which also spread over the Taimyr Peninsula and the Severnaya Zemlya. The area covered by the BIS totalled up to 3.8 Mkm², which was 0.5 Mkm² more than the area covered by the Scandinavian Ice Sheet.

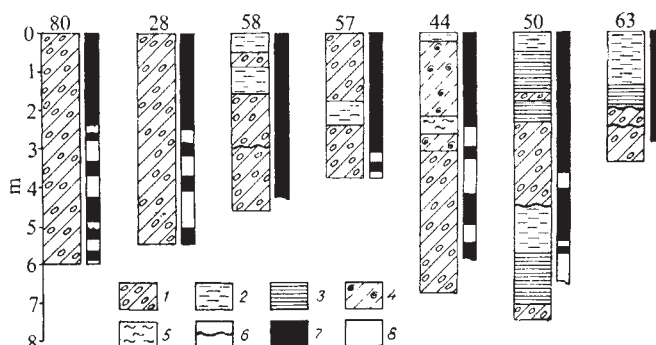
The BIS radically influenced atmospheric circulation. It nearly covered the northern interval between the Scandinavian and the Greenland Ice Sheets. The barrier of ice sheets probably more than 3 km high, ran from the Spitsbergen almost to the Carpatians. Anticyclones constantly dominated the glaciers. All this blocked the altitudinal circulation of the atmosphere in mid-latitudes and caused a decrease of supply to the eastern and southern parts of the Scandinavian Ice Sheet. The most arid conditions were typical of the final stages of the periods of glaciation⁹. Hence, the Scandinavian Sheet began to wane, and between 8,000 and 9,000 yr ago disappeared completely. The glaciers of North America also retreated, although not so rapidly.

Meanwhile, the BIS was waning very slowly (Fig. 1), keeping close to its maximum size until as late as 9,000–9,500 yr BP (ref. 19). On the banks of the lower course of the Pechora-river lake, deposits overlain by a moraine were radiocarbon dated to 9,110 ± 60; 9,250 ± 140; 9,310 ± 60; 9,900 ± 110; 9,900 ± 100 yr BP and other datings falling within the same time interval. The BIS was situated to the north of the polar circle and could have existed up to the present in the same way as the Greenland Ice Sheet does. However, the rise in the ocean level caused by melting of glaciers in mid-latitudes, as well as the isostatic subsidence of the bed of the Barents Sea, intensified the formation of icebergs at the BIS edges, which brought about its rapid disintegration and disappearance.

The Barents Sea appeared at the place of the former BIS. The climate with more profuse snowfalls and cool summers was established in Scandinavia. The conditions were again becoming favourable for a new glaciation. In the absence of man's activity²², a new glaciation might start in the next thousand years. In the past, relatively short interglacial periods were repeatedly followed by long periods of glaciation.

Thus, the BIS may be considered the key to the two main riddles of glaciation. Its existence in late Pliocene and early Pleistocene enables us to understand why glaciation in mid-latitudes did not begin earlier than the end of the Mutuyama epoch. During the Brunhes epoch, the area north of Europe

Fig. 2 Bottom cores from the Barents Sea taken during the 23rd expedition of RV Akademik Kurchatov. The sites where the cores were taken are shown in Fig. 1. 1, till (ancient clays); 2, clays and silts; 3, varved clays; 4, fine loams containing fragments of mollusc shells; 5, lumpy clays; 6, interruption in accumulation; 7, direct polarity; 8, reverse polarity.



was occupied at times by the Barents Sea, stimulating the glaciation of mid-latitudes and at other times the vast ice sheet contributed to the disappearance of glaciers to the south. This factor preconditioned the alternation of glacial and interglacial periods.

D. D. KVASOV
A. I. BLAZHCHISHIN

Academy of Science of the USSR,
Institute of Oceanology,
I liniya 30, Leningrad 199053, USSR

Received 29 December 1977; accepted 13 February 1978.

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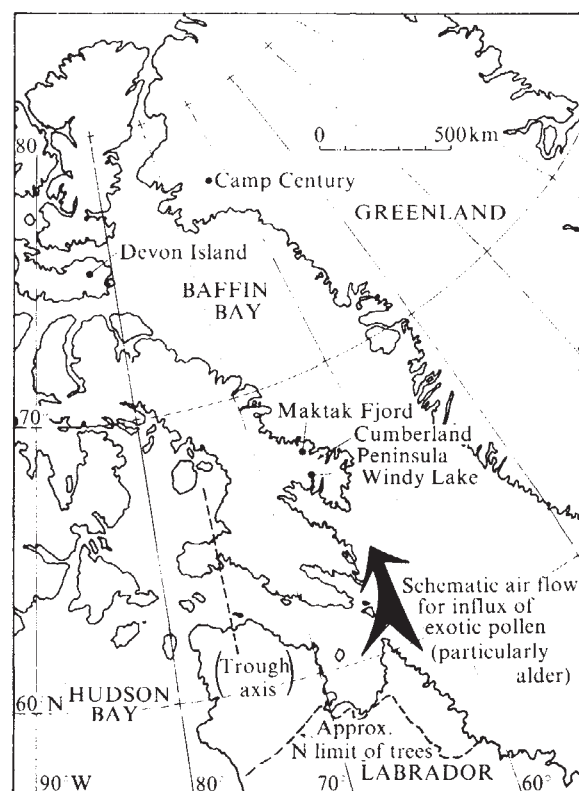


Fig. 1 Location of the two pollen sites on Cumberland Peninsula and relationship to average position of the trough axis and suggested direction of air flow for influx of alder, pine and spruce pollen into Cumberland Peninsula.

Holocene palaeo-wind evidence from palynology in Baffin Island

TRACE amounts of wind-blown tree pollen from the highly productive boreal coniferous forests are a characteristic component of arctic tundra sediments^{1,2}. These exotic pollen are generally regarded as mere contamination or distractions in tundra pollen diagrams³. We report here that traces of exotic pollen in late Holocene sediments from Baffin Island can be used as palaeo-wind indicators, and may be associated with periodic shifts of a trough in the atmosphere.

In tundra sites 550 km from the forest, H.N.⁴ found clear relationships between numbers of tree pollen in Holocene tundra peats and the climatic history of the arctic tree-line to the south. In 1972 and 1973 a line of Tauber⁵ pollen traps was laid down along a 1,600-km transect from tree-line to the high Arctic to record relationships between synoptic meteorology and modern tree-pollen deposition in the tundra⁶; small numbers of pine, alder and spruce pollen (about one exotic pollen grain of each taxa cm⁻² yr⁻¹) were recorded on Baffin Island, more than 1,200 km from their most northerly tree limits.

H.N.⁷ modified the standard pollen counting routines³ to emphasise the exotic tree pollen; using both percentage and 'absolute' dry sediment weight approaches we established suitable totals comprising local, regional and exotic pollen for each sampled level⁷. At that stage of the pollen counts the exotic tree pollen were barely represented, but by prolonged further counting of only the exotic pollen, statistically satisfactory numbers of tree pollen were established and related to the microscope-slide area counted and thus to 'absolute' pollen values.

We have examined sub-fossil pollen grains preserved in late Holocene peats from Cumberland Peninsula in eastern Baffin Island (Fig. 1). This region lies below a major atmospheric standing (Rossby) wave formed by the northern Hemispheric circulation⁸, with an average position of the trough axis at about 75°W longitude. A prolonged shift of the trough to the east or west of its normal position acts as a switching mechanism for airmass trajectories into the region; thus a westward shift

of the trough increases southerly airflow into Cumberland Peninsula, while an eastward shift produces northerly and easterly windflows there. This is interesting because the trough position has significant consequences for past and present status of climate, ice cover, plant and animal life and human occupation in eastern Baffin Island⁹, with likely impacts 'downstream' in Greenland, the north-east Atlantic and elsewhere.

The two monoliths of peat sediments collected by Miller and Boulton were dated by 16 radiocarbon assays¹⁰; the pollen sampling interval was 2.5 cm. Peats may accumulate at irregular sedimentation rates, and may cease to grow at times, but they experience less sediment mixing than many holomictic lakes normally used for palynology; absence of sediment homogenisation is of paramount concern in our study. The rate of peat accumulation at Windy Lake was nearly linear throughout, about 16.5 yr cm⁻¹, from 3,600 yr BP to 850 ± 65 yr BP, while the Maktak Glacier peat covered 2,500 ± 170 yr BP to 1,480 ± 160 yr BP and underwent nonlinear accumulation. Our sampling intervals from both were chosen to average 40 yr.

For the time series these studies were designed to investigate Holocene palaeo-wind vectors by counting exotic tree pollen deposition⁷, and a fuller report on these data is in preparation. The analytical techniques⁷ were not designed to examine fully with the necessary precision the short-term variations or periodicities which have unexpectedly emerged, so that we stress this latter aspect forms only an interim hypothesis, now in process of further testing by closer sampling (1-cm intervals) of other sections.

Our studies of ancient and modern pollen deposition in Keewatin, Mackenzie, Baffin Island and Labrador^{4,7,11,12} suggest that the influx of exotic pollen in these peats was primarily due to winds of southerly origin passing over the boreal forest during the growing season. The exotic pollen peaks are composed of distinctive ratios of high alder to pine pollen (with alder usually an order of magnitude greater than

pine pollen numbers) which for the central and eastern Canadian Arctic are unique matches for north-east Labrador-Ungava both in the Holocene and modern pollen counts. We therefore conclude that the winds responsible for the exotic pollen peaks in the sediments came from due south from their source in Labrador into eastern Baffin Island, a minimum distance from the northernmost pines, spruces and alders of 1,200 km.

There presumably were Holocene variations in tree and shrub pollen production in the source region of north-east Labrador-Ungava due to plant succession, fires, and alterations in growing season climate. We expect the local short-term effects (such as succession and fire) would have been averaged out over the larger region and would not have affected the exotic pollen influx in Baffin Island, but regional variations in pollen production would imply regional climatic change in the source area, of comparable interest to the palaeo-wind changes we infer. Indeed it seems likely that the two effects (productivity changes in the forest of north-east Labrador-Ungava plus wind shifts over eastern Baffin Island) might be a common response to altered strength of the circumpolar vortex.

The exotic pollen record at the two sites is a series of brief episodes of relatively heavy influx of tree pollen ('spikes'), separated by longer intervals with little or no exotic pollen (Fig. 2). We have applied spectral analysis to the numbers of the three main exotic pollen taxa, *Pinus* (pine), *Alnus* (alder), and *Picea* (spruce), to test whether there are underlying periodicities. The possibility that the exotic pollen spikes might be 'noise' has been resolved by a standard significance test of the peaks in the power spectra¹³. For this we used the Fourier transform of the autocorrelation function with smoothing of the resulting estimates¹⁴.

Power spectra of the pine, alder and spruce pollen profiles at Windy Lake exhibit peaks with a period of about 200 yr for all taxa (Fig. 3). The three exotic pollen taxa are statistically independent so that the similarities in the three spectral peaks offers considerable evidence for periodicities, apparently of ~200 yr. Alder, the most numerous of the exotic pollen, exhibits nine maxima in the 2,000-yr record at Windy Lake, with average intervals between spikes of 200–250 yr.

Several studies of late Quaternary and recent quasi-climatic data have identified periodic changes at approximately 200-yr intervals; these include analysis of peat bog growth over 5,500 yr (270-yr periodicity)¹⁵, spectral analysis of ^{18}O fluctuations in

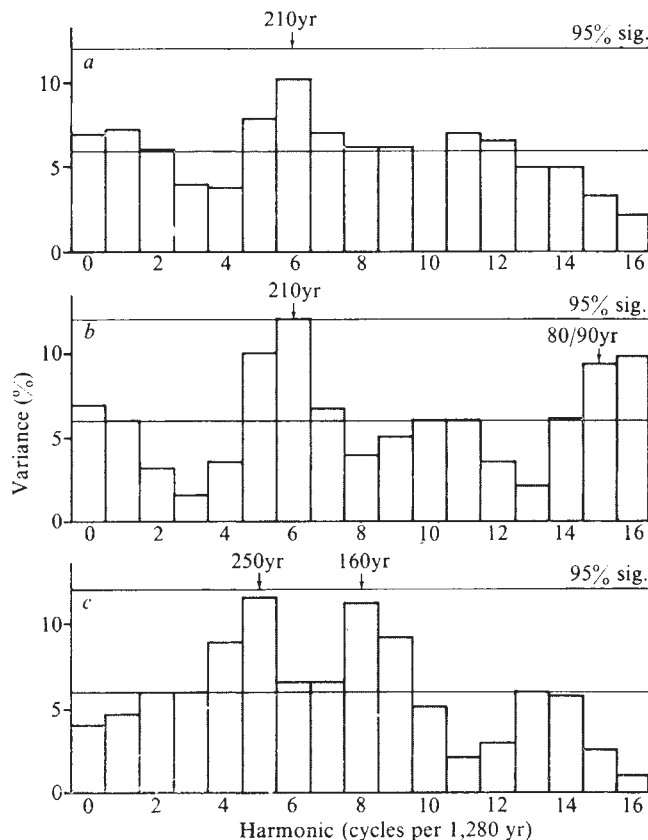


Fig. 3 Variance spectra of pine (a), spruce (b) and alder (c) pollen data for Windy Lake.

ice cores (180-yr periodicity)¹⁶, stepwise changes in reconstructed climatic data for the last millennia^{17,18}, and tree-ring data from Lapland¹⁹ and Japan²⁰; the 200-yr climatic periods have sometimes been compared with volcanic²¹ and solar¹⁷ behaviour. These data are, however, not usually suited to rigorous statistical testing for periodicities of this length. Similarly, the evidence we present here for repeated incursions of southerly air in summer into eastern Baffin Island at approximately 200-yr intervals during the past 2,500 yr is insufficiently established to be used in long-term forecasting, but it is reassuring that these variations correlate well with the history of glaciation on northern Cumberland Peninsula²².

Thus the two major findings of this paper, (1) the ability in at least some geographical areas of determining quite precisely the source of exotic pollen and thus the direction of growing season palaeo-winds, and (2) the possibility of past (and future?) periodicities in these southerly palaeo-winds, are of potential significance for the increasing interest of the climatological community in reconstructing past atmospheric circulation patterns. Our initial findings will be further tested by collection of lake sediments and peats in Baffin Island and in the supposed source region of the exotic tree pollen in Labrador-Ungava.

This work was supported by USNSF grants to H.N., and J. T. A., and NERC fellowship to P. M. K. We thank Susan K. Short for palynological counting assistance, and Drs R. G. Barry, R. S. Bradley, W. Hibley, H. Morth and T. Wigley for their reviews. Contribution to NSF grant ATM77-17549.

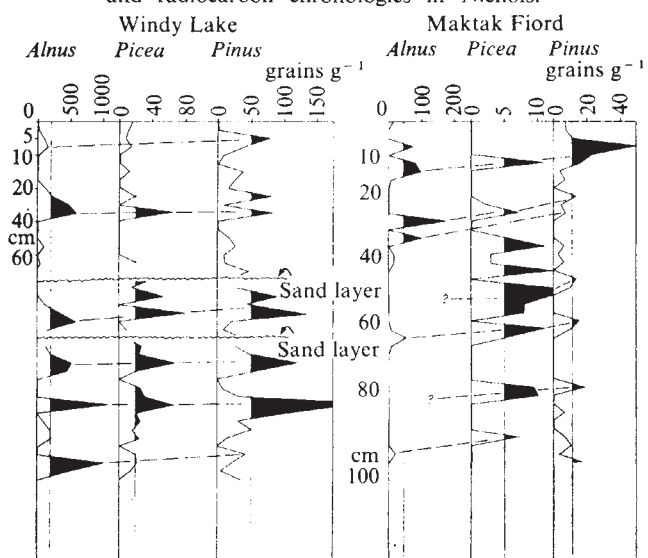
H. NICHOLS

Institute of Arctic and Alpine Research and
Department of E.P.O. Biology,
University of Colorado

P. M. KELLY

Climatic Research Unit,
School of Environmental Sciences,
University of East Anglia,
Norwich, UK

Fig. 2 Counts of *Alnus* (alder), *Picea* (spruce) and *Pinus* (pine) pollen as numbers of pollen grains per g dry weight of sediment from the Windy Lake and Maktak Fiord sites. The dashed lines are suggested correlations between exotic tree pollen influx peaks. The fastest growing sections of the Maktak diagram shows slightly metachronous influx peaks (out-of-phase by ~25 years, explanation in preparation). Full details of stratigraphies and radiocarbon chronologies in Nichols.⁷



J. T. ANDREWS

Institute of Arctic and Alpine Research and
Department of Geological Sciences
University of Colorado
Boulder, Colorado 80309

Received 29 June 1977; accepted 28 February 1978.

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Extreme pollution sensitivity of grasses when SO₂ and NO₂ are present in the atmosphere together

PREDICTIONS of the possible effects of atmospheric pollution on vegetation have usually been based on the results of experiments in which plants were fumigated in the laboratory. For many years it was thought that exposures to SO₂ concentrations below 0.30 p.p.m. were not detrimental to higher plants^{1,2}, which led to the belief that this form of pollution is unlikely to cause any appreciable damage to crops in Britain where, even in heavily populated areas, monthly mean SO₂ concentrations are usually below 0.15 p.p.m., and daily means rarely exceed 0.30 p.p.m. (ref. 3). Recently, however, evidence has accumulated that concentrations of SO₂ below 0.15 p.p.m. can be damaging to plants⁴, casting doubt on the comfortable conclusions drawn from earlier studies. We found that laboratory experiments can underestimate the amount of injury resulting in the field, and report here that combinations of SO₂ with NO₂ give more realistic effects.

There are three reasons for underestimation in the laboratory. First, many experimental fumigations were conducted in chambers in which the air was inadequately stirred. This would have resulted in a slow transfer of pollutants into the leaves because of a high diffusion resistance in the boundary layer. The importance of breaking down the boundary layer resistance to a level comparable with that for leaves out of doors has not generally been recognised. We have shown elsewhere⁹ that the growth of S23 ryegrass was unaffected by 0.11 p.p.m. SO₂ in a wind speed of 10 m min⁻¹, whereas the same concentration in a wind speed of 25 m min⁻¹ caused a reduction in yield of about 30% over a period of 4 weeks. Second, most investigators (including ourselves) have fumigated plants with constant levels of pollutants, using 24-h mean values from field monitoring as a guide to concentration. In the field, and especially near industrial sources, the levels of air pollution are in a continual state of fluctuation and levels up to 10 times the daily mean can occur for periods of 1 h or more. We are currently studying the effects of these 'peaks' of high concentration on plant growth. Finally, experiments in which plants are fumigated with only one pollutant at a time could produce incorrect estimates of the contribution to plant damage in the field where exposure to several pollutants often occurs simultaneously. Most of the combustion processes which generate SO₂ also produce a mixture of nitrogen oxides (NO_x), since high

temperatures cause atmospheric O₂ and N₂ to combine to form NO, which is then slowly oxidised to NO₂. When SO₂ and NO₂ are present in the atmosphere together, their short-term effects on plants have been shown to be at least additive⁶, and sometimes synergistic¹⁰. The effects on plant growth of prolonged exposures to low concentrations of these two ubiquitous pollutants in combination have not, as far as we know, been investigated. We have now carried out some preliminary studies, the results of which suggest that the combined effects of these two gases could be the cause of serious losses in crop production in polluted areas, and that when SO₂ interacts with NO₂ there is a large increase in the toxicity to plants.

Four commonly sown pasture grasses were studied: *Dactylis glomerata* L. var. Aberystwyth S37 (Cocksfoot), *Lolium multiflorum* Lam. var. Milamo (Italian ryegrass), *Phleum pratense* L. var. Eskimo (Timothy) and *Poa pratensis* L. var. Monopoly (Smooth-stalked meadowgrass). Plants were raised from seed in a cold greenhouse and used for experiments 6 weeks after sowing. The fumigation system consisted of four specially designed greenhouses, unheated and rapidly ventilated with ambient air which was purified by passage through activated charcoal filters. Fans carefully placed within each greenhouse created air movement sufficient to reduce the boundary layer resistance around the leaves to a value like that in a grass sward out of doors. Controlled levels of pollutants were added for 103.5 h weekly, clean air being given for the remaining 64.5 h. The concentration of each gas used during the 103.5 h of exposure was 0.11 p.p.m. The treatments, with concentrations calculated as weekly means, were as follows: (1) control, charcoal-filtered air; (2) 0.068 p.p.m. SO₂; (3) 0.068 p.p.m. NO₂; (4) 0.068 p.p.m. SO₂ + 0.068 p.p.m. NO₂. The atmosphere in the control glasshouse was found to contain less than 0.001 p.p.m. SO₂ and NO₂. Fumigations were continued for 20 weeks from October 1976 to March 1977. At the end of the experiment, 10 plants of each species were taken at random from each treatment and leaf areas and total dry weights were determined. Percentage increases or decreases, relative to the controls, were then calculated.

Table 1 shows that when the two pollutants were applied singly, their effects on dry weight varied among the four species. While the yields of *D. glomerata* and *Poa pratensis* were reduced by both SO₂ and NO₂, *L. multiflorum* was not significantly affected by either pollutant alone and *Phleum pratense* was affected by SO₂ only. These reductions in yield were not associated with significant reductions in leaf area, except in the case of *Poa pratensis* exposed to SO₂. Treatment with combined SO₂ and NO₂,

Table 1 Percentage reductions (relative to control) in total dry weights and leaf areas of grasses exposed to atmospheres containing SO₂ and NO₂ alone or in combination for 20 weeks

	SO ₂	NO ₂	SO ₂ + NO ₂	Effect*
	Dry weights			
<i>Dactylis glomerata</i>	40§	22†	78§	S
<i>Lolium multiflorum</i>	5 NS	10† NS	52†	S
<i>Phleum pratense</i>	51§	1† NS	86§	S
<i>Poa pratensis</i>	46§	38§	84§	A
	Leaf areas			
<i>D. glomerata</i>	20 NS	1† NS	76§	S
<i>L. multiflorum</i>	22 NS	1† NS	43†	S
<i>Phleum pratense</i>	11 NS	30† NS	82§	S
<i>Poa pratensis</i>	28†	17 NS	84§	S

Concentrations were as follows: SO₂ 0.068 p.p.m.; NO₂ 0.068 p.p.m. SO₂ + NO₂ 0.068 p.p.m. of each.

* Effect: A, Additive effect of pollutants (that is, there was no evidence of interaction when SO₂ and NO₂ were presented together); S, synergistic effect of pollutants (that is, a statistically significant interaction).

Levels of significance established by analysis of variance of the 2 × 2 factorial experiment: †, *P* < 0.05; ‡, *P* < 0.01; §, *P* < 0.001. ¶ Increase.

however, caused large statistically significant reductions in total dry weight, associated with reductions in leaf area, in all four species. With the exception of *L. multiflorum*, the reductions were greater than 75%. It is interesting that the effects of the two pollutants in combination were greater than their summed individual effects, except in the case of *Poa pratensis* which was the species most sensitive to the individually applied pollutants.

If the toxicity to plants of this mixture of two pollutants had been estimated from their effects when applied separately, it would have been seriously underestimated for three out of four species. In the case of *L. multiflorum* no effect at all would have been predicted from the response to SO_2 and NO_2 when applied alone.

The relative sensitivities of the four grasses were surprisingly different. Although *Phleum pratense* exhibited the greatest reduction in dry weight when exposed to SO_2 , it was one of the species not significantly affected by NO_2 . With such marked differences in response to the three treatments, we suggest that in a mixed sward in the field, different mixtures of SO_2 and NO_2 could result in quite different changes in species composition. Prediction of responses under field conditions would, however, require knowledge of the degree of variation of response within each species, and the capacity for resistant individuals to be selected. Such information could only come from a very much larger investigation.

Many aspects of the response of plants to air pollution are still unresolved, but it is clear that if laboratory experiments are to provide information applicable to field conditions, much more care must be taken over the choice of fumigation conditions. Although the other two points we have enumerated are also important, we suggest that interactions between individual pollutants might represent the largest source of error.

We thank the NERC for financial support, and Mrs P. W. Tabner for technical assistance.

T. W. ASHENDEN
T. A. MANSFIELD

Department of Biological Sciences,
University of Lancaster,
Lancaster, UK

Received 13 January; accepted 17 March 1978.

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Simultaneous occurrence of C_3 and C_4 photosyntheses in relation to leaf position in *Mollugo nudicaulis*

SINCE the discovery of C_4 photosynthesis^{1,2} higher plants have been categorised into C_3 plants, which form 3-phosphoglycerate (PGA) as the first labelled intermediate, and C_4 plants, which form 4-carbon acids as the predominant early photosynthetic products. C_4 plants can also be distinguished by features such as Kranz leaf anatomy, low photorespiration, high photosynthetic efficiency and reduced ^{13}C discrimination³. Although 943 C_4 plants are known to be distributed among 18 families, only 18 genera from 10 families include both C_3 and C_4 plants⁴. Kennedy and Laetsch⁵ described *Mollugo verticillata* as intermediate for C_3 and C_4 photosynthesis. But Brown and Brown⁶ felt that it was a typical C_3 plant. Recently three *Panicum* species

(*P. milioides*, *P. hians* and *P. laxum*) have been found to have photosynthetic characteristics intermediate between C_3 and C_4 plants^{6–8}. We now report that a single plant of *Mollugo nudicaulis* can have some leaves with C_3 characteristics and others with C_4 characteristics according to their position on the stem.

Plants of *M. nudicaulis* L. were collected from their natural habitat on the campus of Sri Venkateswara University (approximately 12-h photoperiod with temperatures of 35 °C by day and 25 °C by night). Plants chosen were about 8 weeks old with unbranched stems each bearing 12–14 leaves, the first being the youngest, fully unfolded leaf. The second to fourth leaves were taken from 4-week-old seedlings of *Oryza sativa* L. variety IR-22, and *Setaria italica* Beauv. variety AKP-2, grown in seed pans in similar conditions.

After preillumination for 30 min in air containing $^{14}\text{CO}_2$, leaves were exposed to $^{14}\text{CO}_2$ for 5 s (at an irradiance of 200 W m^{-2} under tungsten lamps and at 33 °C $\pm$ 1 °C) and their early photosynthetic products were analysed as before⁹. Photosynthetic rates were computed from the total $^{14}\text{CO}_2$ fixed during 5 s. Photorespiratory rates were determined by Kennedy and Laetsch's modification⁷ of Zelitch's method¹⁰. Stomata were opened before the assimilation period by passing CO_2 -free air over the illuminated leaves for 45 min. Air flow was then stopped, and the leaves were allowed completely to assimilate 2 μmol of $^{14}\text{CO}_2$ for 45 min. Then the $^{14}\text{CO}_2$ evolved in the light was compared with the $^{14}\text{CO}_2$ evolved in darkness. The $^{14}\text{CO}_2$ -trapping agent consisted of 50 ml of 1.0 N KOH with 0.6% isoamyl alcohol. Although the pools of photosynthetic intermediates were not estimated during this experiment, the technique of measuring $^{14}\text{CO}_2$ evolution in the light and in the dark was useful for detecting the occurrence of photorespiration in C_3 as well as C_4 plants and has been used as an arbitrary measure of photorespiration^{9,10,11}. Chlorophyll was determined after extracting into 80% acetone¹² and the chlorophyll *a/b* ratio was estimated as before¹³.

Table 1 presents the photosynthetic characteristics of *M. nudicaulis* leaves at different positions on the stem. The features can be compared with those of *O. sativa*, a C_3 plant, and *S. italica*, a C_4 plant. Based on leaf position and photosynthetic characters, we placed them in three categories: young leaves (at positions 1–4 on the stem), old leaves (at positions 11–13 or 14) and middle-aged leaves (positions 5–10).

The young leaves had normal anatomy with palisade and spongy parenchymatous tissue and with no sheath cells around the vascular bundles. On the other hand, old leaves had typical Kranz anatomy with chlorenchymatous bundle sheaths specialised for starch accumulation. Middle-aged leaves had an indistinct chlorenchymatous bundle sheath which did not stain for starch.

There was no regular variation in chlorophyll content (per unit fresh weight) except that the younger leaves tended to have more chlorophyll than did the older leaves. The chlorophyll *a/b* ratio also showed little correlation with leaf age, although the highest ratio was in the 12th leaf.

Young leaves incorporated most of the ^{14}C into PGA and sugar phosphates, and they had lower photosynthetic rates and a higher photorespiratory ratio than did old leaves (Table 1). On the other hand, the old leaves formed mostly C_4 acids (malate and aspartate) as early photosynthetic products, and they had higher photosynthetic rates and a low photorespiratory ratio. The old leaves resembled the leaves of *S. italica* while the young leaves were similar to those of *O. sativa*.

Our data suggested the occurrence of both C_3 and C_4 pathways in leaves of different ages on *M. nudicaulis*: Young leaves were C_3 , old leaves were C_4 and middle-

Table 1 Photosynthetic characteristics of *M. nudicaulis* leaves

Material	Leaf position	Early photosynthetic products*				Photo-synthetic rate	Photores-piratory ratio
		Malate + aspartate	PGA	Sugar phosphates	Others†		
<i>M. nudicaulis</i> Young	1	28	58	8	5	39.8	0.75
	2	26	64	2	8	35.6	—
	3	15	68	5	12	27.8	0.60
	4	18	59	8	15	23.4	—
	5	27	43	12	18	29.3	0.38
	6	32	48	10	10	35.1	—
	7	35	45	8	12	47.7	0.36
	8	42	36	12	10	52.5	—
	9	53	24	8	15	59.8	0.23
	10	60	22	5	13	62.6	—
Middle-aged	11	68	18	2	12	65.8	0.21
	12	72	6	4	18	68.2	—
	13	65	10	5	20	66.4	0.06
Old							
<i>Setaria italica</i>	2-4	81	11	5	3	65.6	0.16
<i>Oryza sativa</i>	2-4	7	62	29	2	29.5	0.43

The data on *S. italica*, a C_4 plant and *O. sativa*, a C_3 plant are given for comparison. The photosynthetic rate is expressed as $\text{mg } ^{14}\text{CO}_2$ fixed $\text{dm}^{-2} \text{ h}^{-1}$ and photorespiration as $^{14}\text{CO}_2$ evolved, light/dark.

*Percentage of total ^{14}C fixed.

†Consisted mostly of alanine.

aged leaves were intermediate. The transition from the C_3 pathway in young leaves to C_4 in old leaves was manifest in at least five features—leaf anatomy, starch staining in the bundle sheath, early photosynthetic products, photorespiration and photosynthetic rate. It is possible that a similar situation exists in *M. verticillata* which has been observed to be a C_3 - C_4 intermediate by Kennedy and Laetsch³ and a C_3 plant by Brown and Brown⁶. Our observation also points to the presence of C_3 and C_4 ecotypes within *Alloteropsis semialata*¹⁴.

During C_4 photosynthesis CO_2 is primarily fixed into C_4 dicarboxylic acids which are later decarboxylated¹⁵. The released CO_2 is then refixed in the Calvin cycle, that is C_3 metabolism. On the other hand, in C_3 photosynthesis CO_2 is fixed and metabolised by the Calvin cycle alone. Thus C_4 plants show an excellent coordination of C_4 acid formation and the operation of the Calvin cycle. Our observations indicate that there is an imbalance in the development of these two systems in *M. nudicaulis* leaves. The C_3 component forms first (in younger leaves) and C_4 system gradually develops later (in older leaves).

G.R. was supported by a CSIR Junior Research Fellowship.

A. S. RAGHAVENDRA
G. RAJENDRUDU
V. S. R. DAS

Department of Botany,
Sri Venkateswara University,
Tirupati 517 502,
Andhra Pradesh, India

Received 11 September 1977; accepted March 23 1978.

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Infanticide and other reproductive strategies in the collared lemming, *Dicrostonyx groenlandicus*

COMPETITION among males for females may continue after mating¹. For example, postfertilisation competition among males occurs in many primates². In rodents, unfamiliar (or strange) males can block pregnancy (Bruce effect) either before³⁻⁹ or after^{7,9} implantation. Here, we report on postparturition effects of unfamiliar males in the collared lemming (*Dicrostonyx groenlandicus*) and discuss the significance of these effects on reproductive strategies in this species.

Experimental animals were derived from stock trapped in Churchill, Manitoba and Eskimo Point, North-West Territories. All animals were weaned at 30 d of age, housed in pairs of the same sex in polycarbonate cages (36×36×16 cm), and provided with corn grit for substrate. Water and guinea pig ration were supplied *ad libitum*, and the temperature ($17 \pm 2^\circ\text{C}$) and the light cycle (22 h light 2 h dark) were controlled. Nulliparous females were kept in these conditions until used in an experiment. Males and multiparous females had bred successfully before the experiment.

To begin each experiment, a female was placed in a clean cage with a male (hereafter referred to as a stud male) for 7 d. The male was then removed, and the female was not disturbed until the end of gestation. At parturition, 80 females with litters were treated in one of five ways (Table 1). In treatments A, D and E, half the females were primiparous and half were multiparous, whereas in treatment B all were primiparous and in treatment C all were multiparous. In treatment A, the young were counted at parturition and were not disturbed thereafter. In treatments B and C, each female and her litter were exposed to an unfamiliar male for 24 h on day 1 *post partum*. In treatment D, each female and her litter were exposed to the stud male for 24 h on day 1 *post partum*, and in treatment E each female and her litter were exposed to a strange male for 24 h on day 3 *post partum*. Interactions were observed

for the first 30 min of each replicate of treatments B, C, D and E.

When the strange male was introduced to the female's cage, she usually attacked him immediately. Continued aggression by the female caused the male to retreat to a corner furthest from the nest. In such cases, no young were killed. One male was killed by a female. If females reduced aggression after their initial attack, males frequently killed the young. Males always killed pups by biting them, usually in the head, and did not cannibalise the dead pups. Females did not kill any young, though occasionally, they licked pups that were bleeding. Strange males killed the young within the first half-hour after they were introduced to the female's home cage. Copulation often occurred during the same period.

When the stud male was introduced to the female's cage, he was also attacked immediately; however, once contact had been made, fighting usually stopped and after a short sniffing bout, the female returned to the nest. The stud male often helped care for the young.

No significant difference ($\chi^2=0.39$; $P>0.05$) was observed in the number of young killed by the strange male between treatments B and C (Table 1). However, a $4 \times 2 \chi^2$ test indicated a significant difference in survival of young when treatments A, D and E were compared separately with treatments B and C together ($\chi^2 = 36.8$; $P<0.001$). No significant difference was found between treatments A, D and E ($\chi^2 = 1.72$; $P>0.05$), but the difference was significant when treatments A, D and E were compared with treatments B and C together ($\chi^2 = 36.1$; $P<0.001$).

Length of gestation of females that were mated *post partum* and had subsequent litters were compared. In eight females in treatments B and C whose first litter suffered no losses due to the unfamiliar male, the gestation period for the *post partum* litter was 28.0 ± 1.0 d (mean $\pm$ s. e.) (range 24–31), whereas in three females whose first litter was killed by the strange male, the gestation period for the *post partum* litter was 21.0 ± 0.6 d (range 20–22). In addition, the mean gestation period of nine females, who mated *post partum* with the stud males, was 22.4 ± 0.7 d (range 20–27). No significant difference was found between length of gestation period of females who mated *post partum* with stud males, and those who mated with unfamiliar males who killed the litter ($t=0.92$; $P>0.05$). There was some level of significance, however, when the preceding two means were compared with length of gestation period of females who mated *post partum* with strange males who did not kill the litter ($t=5.63$; $P<0.001$).

Weaning success of female lemmings was thus significantly reduced when an unfamiliar male was introduced into the nest area on day 1 *post partum*. This decline in weaning success did not occur if strange males were absent, if they were introduced on day 3 *post partum*, or if stud males were introduced on day 1 *post partum*. The effects of unfamiliar males on day 1 did not differ between

primiparous and multiparous females.

During lactation, gestation is reportedly not prolonged more than a few hours in Norwegian lemmings, *Lemmus lemmus*¹⁰ or brown lemmings, *L. trimucronatus*¹¹, but previous studies on *Dicrostonyx* have yielded conflicting results (ref. 12 and R.J.B., unpublished). Relationship of males to females was not indicated in any of these studies. Our data clearly show that delayed gestation occurs in lactating female lemmings when mated with strange males. The problem of delayed gestation in microtines seems to be a phenomenon caused by unfamiliar males, during lactation, rather than a result of lactation itself. We do not know as yet at which stage of the process of gestation the delay occurs, and delayed implantation has not been found in any microtine¹².

The important implications of these results are that there is postfertilisation competition between males and that males and females have conflicting reproductive strategies. Male *Dicrostonyx* not only can terminate a pregnancy sired by another male (F.F.M. and R.J.B., unpublished) but also, may destroy another male's litter. If a male kills the litter and then mates with the female, his offspring will be born after a normal gestation period (21 d). If males do not kill the young, yet mate with the female, then on the average their offspring will be born after a prolonged gestation period (28 d). As lemmings live in the Arctic where duration of the breeding season is a critical factor in reproductive success¹¹, prolonged gestation may have a significant effect on reproductive success. Therefore, the advantages of infanticide by strange males seem multiple and obvious.

The female increases her reproductive fitness if she weans all her litters regardless of which male sired them. At parturition, females have already invested considerable effort into their young and therefore, they should counter the infanticidal tendencies of males. In our experiments, females protected their litters more effectively on day 3 *post partum* than on day 1 *post partum*. In *Mus*, there is a significant increase in female aggression towards males after 48 h of suckling compared with after 24 h, and it seems that suckling is the stimulus that induces this aggression¹³. Prolactin, and possibly progesterone, have been implicated in the hormonal control of this behaviour^{14,15}. It is possible that on day 1 after parturition, the new hormonal regime which maintains *post partum* aggression is not as yet established, and as a result, the litter is more susceptible to male aggression. During oestrus, female *Dicrostonyx* are more ambulatory¹⁶, and field observations have shown that they leave the nest to mate during *post partum* oestrus¹⁷ with mating often occurring outside burrows on the open tundra. As unfamiliar males were placed in the females' cages on day 1 *post partum*, this behaviour was not possible. These observations suggest that females do protect their litters in the wild, actively by aggression, and passively by leaving the nest area to mate.

In *Dicrostonyx*, population density fluctuates greatly from

Table 1 Differences in neonate mortality induced by unfamiliar and stud males, in various treatments

Treatment	Number of litters	Total number of young	Number of young surviving to weaning	Number of young not surviving*	Percentage killed by male
A Control (50% primiparous, 50% multiparous)	16	42	38	4	0.0
B One day <i>post partum</i> (primiparous, unfamiliar male)	16	36	18	18	50.0
C One day <i>post partum</i> (multiparous, unfamiliar male)	16	49	29	20	36.7
D One day <i>post partum</i> (50% primiparous, 50% multiparous, stud male)	16	43	41	2	0.0
E Three days <i>post partum</i> (50% primiparous, 50% multiparous, unfamiliar male)	16	39	34	5	12.8

*Eight pups died before weaning (four in treatment A, two in treatment C, and two in treatment D) which were not killed by males.

year to year. There is still no satisfactory explanation for these 'cycles'^{18,19}. We suggest that the strategies described above may play a significant role in such cycles. For example, it is probable that at low densities, females are unlikely to encounter strange males very often¹⁷, and therefore pregnancy block and infanticide would not be important. The female's strategies, described above, would increase her probability of mating and minimise whatever risk there was of losing her pups to strange males. Given sufficient extrinsic conditions, such populations should increase rapidly. However, as density increases, so does dispersal²⁰ and females would experience increased interference by strange males. This would result in increased incidence of pregnancy blockage²¹, infanticide and prolonged gestation.

The competing reproductive strategies of males would decrease the recruitment of young animals to the population, and ultimately produce a population with a higher proportion of older animals. These density-dependent phenomena would reach a climax during peak years and contribute to a marked decline in the next season's population, due to normal mortality rate in older animals.

Decades of research on rodent populations have concentrated (not too successfully) on elucidating mechanisms which regulate populations, with the assumption that such regulation was the primary function of these mechanisms. By shifting our emphasis to questions relating to the function of competing individual strategies²², we may obtain a fresh perspective and a better understanding of population processes.

We thank J. R. Elliott, D. M. Lavigne, K. Myers and B. Webster for reviewing the manuscript. This work was supported by a National Research Council of Canada grant (No. A5990) to R.J.B.

FRANK F. MALLORY*
RONALD J. BROOKS

Department of Zoology,
University of Guelph,
Guelph, Ontario, Canada, N1G 2W1

Received 31 January; accepted 26 February 1978.

*Present address: Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, Canada, N2L 3C5.

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A paradox in the control of energy intake in the rat

THE precise control of energy intake in rats¹ is generally considered to operate to maintain energy balance. Of the many theories proposed to describe this relationship, the most prominent are the glucostatic², the lipostatic³ and, more recently, the glucolipostatic⁴ theories. All three presuppose that the regulated quantity is related to body energy storage and, therefore, to energy balance. We report here that the control of energy intake can be dissociated from the state of energy balance in the rat, thus demonstrating that

the above supposition is no longer tenable, and leaving the control of energy intake as a physiological enigma.

These three theories of intake control suggest that any experimentally induced perturbation in body energy stores should result in compensatory adjustments to energy intake. To test this prediction, we took advantage of the increase in fat deposition that occurs when a 'nibbling' animal, such as the rat, is forced to consume a large portion of its daily intake in a few discrete meals⁵. The greater weight gain and fat deposition of 'meal-fed' rats occur even when the energy intake is equal to that of free-feeding controls, and is, therefore, due to a greater efficiency of energy utilisation in the meal-fed animal⁶. With this in mind, we fed adult rats various proportions of their energy intake by stomach tube, but allowed normal access to the powdered control diet. Because only a fraction of the *ad libitum* intake was tube-fed, these rats were hypothetically able to reduce voluntary intake to compensate not only for the intragastric induced by tube-feeding.

The energy intake results from four separate experiments (Table 1) indicate that the first part of this hypothesis certainly holds true. The fraction of the total energy intake fed by stomach tube ranged from 35 to 75% of control levels, and yet in all instances voluntary intake was depressed to the extent that the total intake was precisely the same in both control and experimental animals. This confirms the rat's ability to maintain a constant intake of food, but also for the greater rate of energy retention in spite of severe modifications to its dietary regimen. This ability, however, seems to be totally unrelated to the regulation of energy balance, as the tube-fed animals all gained significantly more weight than controls (Table 1). *In vivo* measurements of body water (by tritium dilution) indicate that most (80%) of this weight gain can be attributed to fat deposition.

The weight gains of control animals are well within the normal range for adult Sprague-Dawley rats eating a well-balanced diet, and thus the relatively greater body weight and fat gains of tube-fed rats represent the development of an obese state. Because the energy intakes of control and tube-fed rats are the same, this obese state must result from an increased metabolic efficiency (that is, a decreased heat production). If gross energetic efficiency had not increased, but had remained the same as control values, the extra fat gain of tube-fed rats would have required an additional intake ranging from 1,100 to 2,000 kJ per rat in the four experiments shown in Table 1. Because energy intake was measured to the nearest 10 kJ d⁻¹, (that is, accurate to within 3%) there can be no doubt that the greater energy retention of the tube-fed animals was achieved on the same input of energy as their controls, and confirms the known effects of meal-feeding on metabolic efficiency.

The precise compensation of voluntary intake after the administration of gastric loads is worth comment because there are several contradictory reports in the literature. Where compensation has been observed, the amounts tube-fed have usually been small and/or given as a single gastric load, whereas incomplete compensation for gastric loading is usually seen when multiple loads, or continuous infusions, of a single nutrient are given (see ref. 7 for review). It seems, however, that nobody has investigated the effects on the residual voluntary intake of rats tube-fed significant amounts of a nutritionally balanced diet for more than a few days, and our results show that in these conditions compensation is precise. In view of this, earlier conflicting reports could be resolved if one takes the view that although small, single-nutrient loads produce compensation of voluntary intake, the rat cannot compensate for multiple loads over several days unless the food delivered intragastrically constitutes a balanced diet. This view is corroborated by the results of an experiment in which significant amounts (34% of energy intake) of a single

Table 1 Effect of tube-feeding on body weight gain and energy intake

Expt	No. of animals	Duration of experiment (d)	Body weight gain (g)	Energy intake (kJ per rat)	Energy intake delivered by tube (% control intake)	Total energy intake (% control intake)
1 Control	6	30	44 ± 5*	11,340 ± 110		
Tube-fed	5		71 ± 10†	11,650 ± 140 (NS)	34.3	103.0
2 Control	12	21	67 ± 5	8,500 ± 110		
Tube-fed	8		100 ± 9‡	8,400 ± 130 (NS)	47.5	99.7
3 Control	6	23	58 ± 5	8,860 ± 90		
Tube-fed	6		95 ± 5‡	8,420 ± 80 (NS)	68.2	95.3
4 Control	12	21	40 ± 5	7,700 ± 30		
Tube-fed	9		89 ± 5§	7,710 ± 50 (NS)	74.7	100.0

Tube-fed animals received one (Expt 1), two (Expt 2) or three (Expts 3 and 4) daily intragastric loads of Complan (Glaxo) mixed into a slurry (dry weight of slurry varied over 30–60% w/v). All animals had free access to a semi-synthetic powdered diet with a nutrient composition similar to that of Complan. Animals were all adult male Sprague-Dawley rats, weighing 300–450 g. NS, not significant.

*Mean ± s.e.m.

† $P < 0.05$

‡ $P < 0.01$

§ $P < 0.001$

nutrient (fat) were tube-fed for 30 d. Irrespective of the type of fat (saturated animal fat or unsaturated vegetable oil), the rats failed to compensate for the energy delivered by tube and ate 15–16% more food than controls (Table 2). The most likely explanation of the incomplete compensation for multiple loads of single nutrients is that the rats overeat in order to obtain a sufficient intake of other nutrients.

Table 2 Effect of tube-feeding fat on energy intake

	Energy delivered by tube (kJ)	Total energy intake (kJ)	Energy intake (% control)
Control	—	11,340 ± 110*	100
Tube-fed (butter)	3,850	13,200 ± 160†	116
Tube-fed (corn oil)	3,850	13,000 ± 500†	115

Rats were tube-fed 34% of control energy intake as fat (butter or corn oil) for 30 d. All rats had free access to a semi-synthetic powdered diet.

*Means ± s.e.m. ($n = 6$ in each group).

†Significantly different from controls, $P < 0.01$.

Having shown that a balanced diet given intragastrically does not impair the rat's ability to maintain a constant energy intake, one is left with the paradox that this control is independent of the metabolic fate of the ingested energy and, in consequence, the regulation of energy balance is rendered defective and obesity results. Clearly, this control cannot be explained on the basis of existing theories, which regard the body energy content as the fundamental determinant of energy intake. If the size or rate of accretion of body energy stores do not determine energy intake, the question of what does is enigmatic. It has been established that rats do not normally eat for bulk¹, and because the dry weights of the gastric loads used in our experiments varied from 30 to 60%, we support this view. An alternative is that the rats were eating for a specific nutrient or nutrients, but we have no evidence for this, other than that inferred from experiments where nutritionally unbalanced loads were given intragastrically.

It might be thought that the energostatic theory of feeding described by Booth and Toates^{8,9} could account for an independence of intake control from energy balance, as this model of feeding is based on energy supply and availability and not on the set-point regulation of any specific body energy store. Unfortunately, this model is based on short-

term responses to variations in intermediary metabolism and the effect of body energy increments has yet to be integrated into the model. Even as the model stands, it is difficult to conceive an explanation of our results, given that the energy supply to metabolism is in excess of energy requirements and should therefore result in a depression of voluntary intake rather than the sustained deposition of the excess energy as fat.

Although tube-feeding can, itself, introduce complications into any study of feeding behaviour and metabolism, we suggest that the uncoupling of intake control from energy balance reported here could occur in other situations and that conclusions drawn from feeding experiments must take into account changes in energy retention if they are to be integrated into existing ideas on the regulation of energy balance.

NANCY J. ROTHWELL
M. J. STOCK

Department of Physiology,
Queen Elizabeth College,
Campden Hill Road,
London W8, UK

Received 21 December 1977; accepted 29 March 1978.

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Placental inhibition of foetal growth enhancement in the rat

STUDIES in the rat have demonstrated that surgical reduction of litter size (LR=litter reduction) in the first half of pregnancy results in growth enhancement of the surviving placantae and foetuses at term^{1–3}. In addition to providing a useful preparation for studying relationships between structure and function during development, this technique may offer some insight into the mechanisms which regulate foetal and placental growth. We observed recently that LR at a later stage of pregnancy (day 13) produced no increments in either placental or foetal weight in the survivors³. At this time, the operation seems to be more traumatic for the mother, and this may

Table 1 Effects of removal of all embryos except one (group 1), removal of all embryo-placental units except one (group 2) and the sham operation (group 3) at day 13 on maternal adrenals and ovaries at day 21

Group	No. of mothers		Pregnant at term	No. of conceptuses at day 13*	Adrenal weight (mg)*	Ovary weight (mg)*	No. of corpora lutea*
1	14	11	8	11.83±0.31	66.2±2.6	119.1±6.5	16.17±0.60
2	16	14	5	9.80±1.60	64.2±2.6	88.6±6.2†	17.60±2.10
3	9	9	7	9.33±2.10	66.9±5.4	127.1±9.1	18.66±0.88
			<i>F</i> ratio	0.77	0.13	6.83	1.07
			<i>P</i>	NS	NS	<0.01	NS

Results of one-way analysis of variance shown below. Data from one group 3 mother which had delivered before sampling, and two group 1 mothers which had dead foetuses are not included. NS Not significant.

* Mean±s.e.m.

† Significantly less than respective control value ($P < 0.01$).

account for our failure to observe growth enhancement. Alternatively, the mechanisms which regulate growth may be impervious to influence at this later stage. However, an additional feature of LR at day 13 in our previous study was the survival of several placentae without foetuses. The chorio-allantoic or true placenta is formed in mid-pregnancy, and LR after this time may result in foetal death without placental death¹. Apparently, such (supernumerary) placentae retain their endocrinological integrity³, and we have suggested that their presence may inhibit the expression of growth enhancement in surviving foeto-placental units³. We distinguish here between these various possibilities experimentally by reducing the litter size at day 13 both with and without the survival of supernumerary placentae. The results strongly suggest that these placentae are responsible for inhibition of placental and foetal growth enhancement.

Virgin hooded rats of the Lister strain, maintained on a 12-h white light/12-h red light cycle, were mated; the presence of sperm in the vaginal smear defined day 0 of pregnancy. On day 13, subjects were anaesthetised and the uterus exposed. One of three treatments was then carried out. In group 1, all the embryos except one were removed, and no placentae were removed. A small incision was made in the antimesometrial side of each implant, and the amniotic sac, containing the embryo, was exteriorised by slight bilateral pressure. The sac was pushed free, removed, and the opening closed with silk sutures. In group 2, all the embryos and placentae were removed from the uterus except one with its placenta, which was left *in situ*. Because of the known effects of position in the uterus on foetal

development⁶, the position of the single survivor in these two groups was systematically varied. In group 3, a control group, all the embryos and placentae were left intact, but the uterus was traumatised by placing sutures in the uterine wall between each implant and at the cervical and ovarian ends of the uterus. Following these operations, the uterus was returned to its normal position in the abdominal cavity and the wound closed. Mothers in groups 1 and 2 were invariably sickly and listless immediately following the operation, and five died within 48 h. Mean litter size at the time of the operation in the mothers which continued their pregnancies was comparable between the three groups (Table 1).

The effects of these treatments were assessed on caesarean-delivered foetuses and their placentae on day 21, shortly before the expected time of normal delivery. Maternal adrenal and ovarian weights and the number of corpora lutea were also recorded. Placental DNA was extracted by the method of Zamenhof *et al.*⁷, and estimated colorimetrically by a modification of Burton's method⁸ for all placentae of the survivors in groups 1 and 2, and for approximately half of all control and supernumerary placentae.

Ovarian weight was significantly reduced in group 2 (Table 1). The effect was not due to any differences in the number of corpora lutea between groups. Adrenal weight was unaffected by the treatments. Foetal body and brain weights in group 1 singletons with supernumerary placentae were within the normal range (Table 2). However, in group 2 singletons these values were significantly larger than those of the controls of group 3. Placental weight and DNA

Table 2 Effects of the experimental treatments on placental and foetal development at day 21

Group	Weight (g)*	Placenta Total DNA (μmol)*	Concentration (μmol g ⁻¹)*	Foetus Body weight (g)*	Brain weight (mg)*
1	0.4195±0.0258	3.2829±0.5393	8.2205±0.9604	4.5082±0.1853	188.6±4.7
(supernumerary)	0.2144±0.0043†	1.9178±0.0743†	9.1143±0.3050		
2	0.7503±0.0676‡	6.3571±0.5004§	8.6601±0.8098	6.0080±0.1414†	207.9±3.5
3	0.5024±0.0267	3.9593±0.1140	7.4382±0.5325	4.7260±0.1502	184.8±7.4
Increase (group 2 over 3)	49%	61%		27%	13%
<i>F</i> ratio	24.37	24.95	1.06	23.14	4.44
<i>P</i>	<0.0001	<0.0001	NS	<0.0001	<0.05

* Mean±s.e.m.

† Compared with control values, $P < 0.0001$.

‡ $P < 0.025$.

§ $P < 0.005$.

|| $P < 0.05$.

content (representing total nuclear number) were also increased in group 2. The supernumerary placentae in group 1 comprised 91% of the placentae from which embryos had been removed at day 13. They were less than half the normal weight and contained significantly less DNA than normal placentae. In control mothers, there were two supernumerary placentae in one mother and one in another (mean weight 0.2513 g). These resulted from foetal resorptions after day 13, either spontaneously or as a consequence of operation trauma. Placental DNA concentration (representing cellularity) was comparable between normal and supernumerary placentae and was unchanged by any of the treatments.

Our findings demonstrate that neither the increased trauma associated with opening the uterus, nor the later stage at which LR is carried out, preclude the expression of growth enhancement in the surviving placenta and foetus. It has been suggested that growth enhancement is a consequence of reduced uterine crowding, which results in an improved blood supply, providing foetuses with more oxygen and nutrients and removing waste products more efficiently^{1,2,9}. However, the absence of growth enhancement in group 1 singletons suggest that crowding is not the limiting factor. It might be argued that group 1 mothers were exposed to a relatively greater metabolic demand to support a greater absolute mass of foetal and placental tissue due to the presence of supernumerary placentae. However, the mean conceptus mass was only a little greater in group 1 than in group 2 (6.96 g and 6.76 g, respectively). Further, foetal and placental growth in group 1 was comparable to that in group 3, in which the mean conceptus mass was 32.76 g.

These results, together with other recent observations, support the view that placental and foetal growth in the rat is also under hormonal rather than merely mechanical or maternal nutritional regulation. In group 1 of the present study, where total placental mass is reduced but the number of placentae is approximately normal, development of the single foeto-placental unit is normal, suggesting that ovarian output of oestradiol is in the normal range. It is this which normally inhibits placental growth¹⁰, plasma oestradiol levels being inversely related to placental weight¹¹. In contrast, where the number of placentae is reduced to one (group 2), placental luteotrophic support¹² would be considerably less, and this is reflected here in reduced ovarian weight. A concomitant reduction in circulating oestradiol would reduce the normal inhibition of placental growth, resulting in placental hypertrophy and foetal growth enhancement.

Thus, we confirm that foetal growth enhancement may be reliably produced by LR in the rat as late as 13 d of gestation, and is not due to the increase in absolute room in the uterus. The results suggest instead that placental hypertrophy and the associated increase in foetal weight that result from a reduction in litter size will occur only when there is a reduction in the total number of placentae, so that hormonal relationships between the placenta and ovary are modified.

This research was supported by grants from the MRC and the National Fund for Research into Crippling Diseases. P.G.C. was supported by a postdoctoral fellowship from the Canadian MRC. The technical assistance of Stanton Howarth is acknowledged.

P. G. CROSKERRY
JOHN DOBBING

Department of Child Health,
The Medical School,
University of Manchester,
Oxford Road, Manchester, UK

Received 6 October 1977; accepted 31 March 1978.

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Activation of mammalian oocytes by intracellular injection of calcium

OOCYTES of many species undergo parthenogenetic activation after exposure to the calcium ionophore, A23187, even in the absence of external Ca^{2+} (refs 1 and 2), whereas those of mouse, rat and hamster will activate simply on exposure to Ca^{2+} free medium^{3,4}. This suggests that Ca^{2+} is involved in parthenogenetic activation. Direct evidence of a transient increase in intracellular free Ca^{2+} at the time of fertilisation or activation of fish⁵ and sea urchin eggs⁶ has been obtained using the photoprotein aequorin. We report here that parthenogenetic activation and subsequent normal development can be induced in mouse oocytes by injecting them with Ca^{2+} .

Mouse oocytes were prepared for injection as described in Table 1. We used a single microelectrode to either record or pass current. Microelectrodes were filled with 0.2 M CaCl_2 or the chloride salt of Sr^{2+} , Ba^{2+} , Mg^{2+} , La^{3+} , K^{+} or Na^{+} . Cell penetration was indicated by a negative resting potential; when recorded with a microelectrode filled with 3 M KCl the resting potential of oocytes removed from females 16 h after injection with human chorionic gonadotrophin (HCG) was -14.04 ± 0.51 mV (inside negative, $n = 89$). All cations were injected iontophoretically with 1-s pulses of 10–20 nA; the total iontophoretic current injected into any oocyte was within the range 100–150 nC. Iontophoretic injection was chosen in preference to pressure injection to allow for use of micropipettes with a much narrower tip ($\leq 0.5 \mu\text{m}$). In view of the small diameter of the mouse oocyte ($\sim 70 \mu\text{m}$) this minimises the possibility of activation induced by penetration⁸. Simple penetration of oocytes, 16 h after HCG, with microelectrodes of the type used for injection did not result in activation (4/117–3.4%).

Table 1 shows the effect of increasing the intracellular level of Ca^{2+} by injection. When corrected for spontaneous activation, the levels of parthenogenetic activation induced in oocytes 16 h after HCG by Ca^{2+} -injection, and by exposure to Ca^{2+} -free medium⁴ (normal culture medium minus Ca^{2+}), were 46.2% and 43.1%, respectively; the distribution of individual results was the same for both treatments. The similarity of the results for the two treatments suggests that exposure of oocytes to Ca^{2+} -free medium leads to an increase in intracellular free Ca^{2+} or that activation is produced by a secondary effect common to both treatments. Removal of extracellular calcium causes cells to accumulate Na^{+} (ref. 9); in accordance with this, oocytes incubated in Ca^{2+} -free medium for 1 h became depolarised (resting potential = -7.69 ± 0.35 mV, $n = 123$). Because the membrane of the mouse oocyte is excitable in certain conditions and can carry an inward Ca^{2+} current¹⁰, there may be a Na–Ca exchange mechanism analogous to that described for nerve membranes¹¹. If so, injection of Ca^{2+} could also cause an increase in intracellular Na^{+} (ref. 12). However, preliminary observations suggest that Na^{+} injected iontophoretically (100–150 nC) does not activate mouse oocytes.

Because the input resistance of mouse oocytes is about $18 \text{ M}\Omega$ ¹³, even the relatively small current pulses delivered in our experiments (10–20 nA) would have produced a large depolarisation of the plasma membrane (180–360 mV). It is unlikely that this would produce a Ca^{2+} influx, for in the unfertilised mouse oocyte Ca^{2+} spikes are only seen as off-responses after sub-

Table 1 Effect of intracellular iontophoretic injection of Ca^{2+} on activation of MFI oocytes of various post-ovulatory ages

Time of oocyte removal from females (h after HCG injection)	No. of oocytes injected	No. of oocytes activated	% Activation	% Activation of non-injected oocytes
13.30	37	6	16.22	0.00 (0/39)
16.00	225	112	49.78	3.61 (12/332)
20.00	88	78	88.64	45.00 (18/40)

Oocytes were obtained from random bred albino females (MFI, Olac, Bicester, UK) induced to superovulate by the intraperitoneal injection of 5 IU of pregnant mare's serum gonadotrophin (PMS) followed 44–48 h later by an injection of 5 IU of HCG; ovulation in these mice is completed approximately 12.5 h after injection of HCG (unpublished results of D.G.W.). Oocytes were released from the oviducts, at various times after injection of HCG, into mouse embryo culture medium⁷, and the cumulus cells were removed with hyaluronidase (150 U ml⁻¹). Oocytes were transferred to two droplets (50–100 μl) of a modified culture medium under paraffin oil; this medium, containing a reduced amount of bicarbonate (4.15 mM) and HEPES buffer (20.85 mM), allowed the cells to be maintained at constant pH in an atmosphere of air during the period of intracellular injection. The temperature of the droplets was maintained at 35–37 °C by a jacket containing circulating water. The oocytes in one droplet were injected, while those in the other acted as a control for spontaneous activation. During injection oocytes were secured by a small suction pipette (internal tip diameter 10–15 μm), the tip of which had been fire-polished. Iontophoretic injection of a given batch of cells was completed 60–90 min after placing in culture, whereupon both control and injected oocytes were incubated in air at 37 °C for a further 6 h before examination for activation. The criterion for activation was the presence of one or two pronuclei. Data shown for each post-ovulatory age represent the combined results of several experiments.

stantial membrane hyperpolarisation¹⁰. To exclude the possibility that activation was induced by either injection of current or the related membrane depolarisation during Ca^{2+} -injection, similar injections were made 16 h after HCG by means of a KCl electrode. Of 98 cells injected only two had pronuclei after 6 h in culture (2.0%), compared with three in a control sample of 136 uninjected oocytes (2.2%).

Ca^{2+} -injection of oocytes removed from mice 20 h after HCG produced a higher rate of activation than in those removed 16 h after HCG. However, after correction for spontaneous activation, which also increases with age after ovulation⁴, this value (43.6%) did not differ significantly from that observed at 16 h after HCG. MFI oocytes injected with Ca^{2+} ~1 h after completion of ovulation showed a smaller response (Table 1); oocytes of the same post-ovulatory age exposed to Ca^{2+} -free medium also exhibit a much reduced response⁴.

Table 2 shows the effects of injecting other divalent cations into mouse oocytes. Mg^{2+} , injected in amounts similar to Ca^{2+} , failed to induce a significant level of activation, ruling out the possibility that the response to Ca^{2+} resulted from some nonspecific action of a divalent cation. Sr^{2+} and Ba^{2+} produced effects which were not significantly different from those of Ca^{2+} . The relative effects of these divalent cations on activation are in accordance with their actions on other Ca^{2+} -dependent phenomena, for example, the release of neural transmitter substances¹¹ and the permeability of certain membranes¹⁵, and may reflect their comparative abilities to act on Ca^{2+} -binding sites¹⁶. We also have evidence (unpublished) that La^{3+} , which can displace Ca^{2+} from membranes¹⁷, promotes activation of mouse oocytes when added to their culture medium. Intracellular injection of La^{3+} 16 h after HCG failed to induce activation. However, because the transport number for La^{3+} may be very low, cytoplasmic precipitation probably prevents any small amount injected from reaching intracellular Ca^{2+} -binding sites.

Although we could not measure the increase in concentration of intracellular free Ca^{2+} during Ca^{2+} -injection, some estimate can be made of the change in total concentration of intra-

cellular Ca^{2+} before sequestering by intracellular elements. If the volume of the mouse oocyte is ~200 pI and the mean transport number for Ca^{2+} is ~0.1¹⁸, injection of 100–150 nC of Ca^{2+} will raise the intracellular Ca^{2+} concentration by 250–375 μM (see ref. 19 for details of calculation). The resting level of intracellular free Ca^{2+} in mouse oocytes is unknown, but in the sea urchin egg it is believed to be much less than 1 μM (ref. 6). The site of intracellular Ca^{2+} -injection is important in inducing the breakdown of the germinal vesicle and the release of cortical granules in the larger oocytes of amphibians^{20,21}, and in sea urchin eggs the transient Ca^{2+} flux seen at fertilisation is believed to be confined to the cortical layer⁶. In our study the microelectrode tip could never be more than 70 μm from any point within the cell, a distance much smaller than those shown to be critical for other eggs²⁰. The size of the mouse oocyte may thus be advantageous for studies of this nature. The observation, from ultrastructural studies, that cortical granule breakdown occurred in Ca^{2+} -injected mouse oocytes (D.G.W., B.P.F. and E. Anderson, in preparation) may indicate some increase in intracellular Ca^{2+} at the cell surface.

To determine the developmental potential of oocytes activated by Ca^{2+} -injection we examined briefly the *in vitro* development of injected oocytes from F₁ (C57BL $\times$ CBA) females. Twelve out of thirteen (92.3%) injected oocytes developed to the pronuclear stage as compared with 0/10 for noninjected cells. Two of the 12 activated oocytes (16.7%) developed normally through the pre-implantation period, reaching the expanded blastocyst stage (Fig. 1). Of the remaining 10, two developed to the eight-cell stage or beyond, five to the four-cell stage and three to the two-cell stage.

Our experiments show that increasing intracellular free Ca^{2+} in an oocyte by injection induces the normal sequence of events known to follow fertilisation: cortical granule breakdown, release of the oocyte from meiotic block at metaphase II and subsequent cell division. Cortical granule release is recognised as a form of Ca^{2+} -mediated exocytosis²², but the relationship between intracellular Ca^{2+} and the resumption of meiosis is more obscure and it should be borne in mind that the relevant

Table 2 Effect of iontophoretic injection of divalent cations on the activation of MFI oocytes

Ion	No. of oocytes injected	No. of oocytes activated	% Activation	% Activation of non-injected oocytes
Ca^{2+}	225	112	49.78	3.61 (12/332)
Mg^{2+}	67	3	4.48	3.33 (3/90)
Sr^{2+}	79	40	50.63	3.60 (5/139)
Ba^{2+}	76	30	39.47	3.64 (4/110)

Oocytes were removed from MFI mice 16 h after HCG injection. Subsequent treatment was as described in Table 1. Data shown for each ion represent the combined results of a number of experiments.

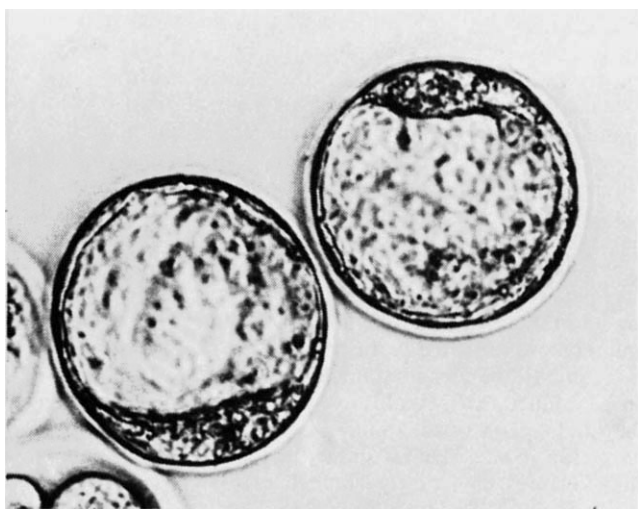


Fig. 1 Blastocysts developed *in vitro* from mouse oocytes injected intracellularly with Ca^{2+} . Oocytes were collected from F_1 (C57BL X CBA) females as described in Table 1 4.5 h after completion of ovulation. After intracellular injection of 100–150 nC Ca^{2+} , oocytes were transferred to droplets of standard mouse embryo culture medium and incubated for 96 h at 37°C in an atmosphere of 5% CO_2 in air. Magnification $\times 650$.

trigger may be some secondary effect of Ca^{2+} -injection such as a change in intracellular pH ^{12,23,24}. The demonstration that mouse oocytes can undergo successful parthenogenetic development after activation by intracellular Ca^{2+} -injection is further evidence that the primary trigger for activation is probably the transient Ca^{2+} flux known to occur in other eggs at fertilisation^{6,8}. For further analysis of the role of Ca^{2+} in activation, intracellular injection may be a more satisfactory method of raising intracellular Ca^{2+} concentration than exposure to a calcium ionophore.

We thank Dr A. McLaren and Professor R. Miledi for reading our manuscript, and the departments of Biophysics and Physiology, University College London for the loan of equipment. D.G.W. acknowledges NATO for additional support.

BARBARA P. FULTON*
D. G. WHITTINGHAM

MRC Mammalian Development Unit,
University College London,
Wolfson House, 4 Stephenson Way,
London NW1, UK

Received 30 November 1977; accepted 13 February 1978.

*Present address: Department of Biophysics, University College London, Gower Street, London WC1, UK.

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β -D-xylosides cause abnormalities of growth and development in chick embryos

THE notion that the composition of the intercellular matrix plays an important part in vertebrate morphogenesis has received considerable indirect experimental support. At least four types of collagen are synthesised and secreted in different proportions by cells from various tissues¹, and many tissues have their own characteristic spectra of glycosaminoglycans, which undergo precise changes during development^{2,3}. Differentiation of several tissues and organs *in vivo* or *in vitro* is accompanied by major changes in collagen and glycosaminoglycans^{4,5}, and can even in some cases be promoted by addition of collagen^{6,7} or glycosaminoglycans^{8,9} to the culture medium. Direct evidence that the structure of collagen influences morphogenetic events is provided by studies *in vivo* with lathyrogens such as β -amino-propionitrile, which interfere with the crosslinking of collagen by inhibiting the action of the enzyme lysyl oxidase¹⁰. When administered to developing embryos or young animals, these agents produce a series of abnormalities including multiple bone deformations, vascular lesions and other manifestations of connective tissue damage¹¹. To date, there have been no similar studies of the role of chondroitin sulphate or other glycosaminoglycans in development, because no agent has been found that specifically affects the synthesis or structure of any proteoglycan *in vivo*. We report here that administration of β -D-xylosides to 9-d-old chick embryos leads to marked alterations in the structure of chondroitin sulphate and simultaneously causes reproducible changes in growth and morphology, which persist at least until the chick is ready to hatch. The morphological changes are unlike those associated with lathyrism.

Biosynthesis of chondroitin sulphate occurs by stepwise transfer of sugar residues to the nonreducing ends of the growing chains, which are covalently attached to a peptide core through a linkage region with the structure glucuronyl- β (1,3)-galactosyl- β (1,3)-galactosyl- β (1,4)-xylosyl- β (1,3)-serine¹². The enzyme that catalyses the transfer of the first galactosyl residue can use several artificial β -D-xylosides as substrates¹³, thereby priming the synthesis of chains of chondroitin sulphate attached to aglycones that are not peptides¹⁴. When chick embryo sterna are incubated *in vitro* with 4-methylumbelliferyl- β -D-xyloside (MUX) or *p*-nitrophenyl- β -D-xyloside (NPX), they synthesise chondroitin sulphate covalently linked to the artificial aglycone, as well as chondroitin sulphate linked to the normal peptide core¹⁵. The chains of both species of chondroitin sulphate are about 25% shorter and 30% less sulphated than the chondroitin sulphate synthesised in the absence of β -xyloside^{15,16}.

Fertilised eggs were obtained from inbred White Leghorns (Spring Lake Farm, Wyckoff, New Jersey). 4-Methylumbelliferone and its glycosides were suspended in 0.1 ml corn oil; derivatives of *p*-nitrophenol were dissolved in 0.3 ml saline. Drugs were administered to 9-d-old embryos (stage 35 of Hamburger and Hamilton¹⁷) by injection into the amniotic sac. Embryos were maintained in a humidified incubator at 38°C until they were killed.

Figure 1a shows embryos that had received 10 mg MUX on day 9 and were killed on day 16. By comparison with control embryos that had received no drug, the treated embryos show generalised oedema, manifested as distension of the abdomen, cranial distortion, pendulous jowls and puffing of the limbs. Because of the distension of the abdomen, the medial and lateral feather tracts on the ventral surface, which are separated by 1.5–2.5 mm in normal 16-d-old embryos, are 4–5 mm apart in treated chicks (Fig. 1a). A similar separation of feather tracts is evident on the dorsal surface. At least 80% of the treated chicks show

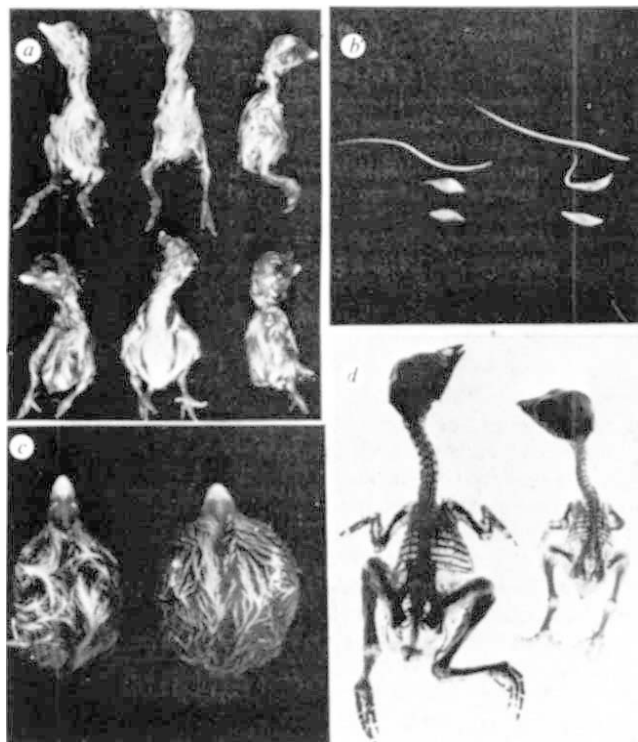


Fig. 1 *a*, 16-d-old control embryos (top row) or embryos treated with 10 mg MUX on day 9 (bottom row). *b*, Feathers from a 16-d-old control embryo (top row) or from two embryos treated with 10 mg MUX on day 9 (centre and bottom rows). Feathers were plucked from the ventrolateral surface of the neck (right) or the medial abdominal surface (left) of embryos that had been dehydrated in 95% ethanol; the feather root is to the right. *c*, Top view of the head of a control 16-d-old embryo (left) and an embryo treated with 10 mg MUX on day 9 (right). *d*, Skeletons of a newly hatched (21-d) control chick (left) and a 23-d-old embryo (right) that had been treated with 10 mg MUX on day 9, stained by the KOH Alizarin red S technique¹⁸.

abnormal morphology of many of the feathers, particularly on the abdomen and ventrolateral surface of the neck. The feather filament is short and distended along most of its length, and retains this shape after fixation in ethanol (Fig. 1*b*). Because we had the impression that treated chicks have fewer feathers than do control embryos, we counted the feathers originating from the upper right eyelid of three MUX-treated, three NPX-treated and six control embryos. The counts in the xyloside-treated embryos ranged from 0 to 14 (mean 9.5), while those of the control embryos ranged from 24 to 36 (mean 29.2).

On superficial examination, the beaks of treated embryos seem short and blunted; this is especially marked when the head of the embryo is viewed from above (Fig. 1*c*). Measurements of the length of the upper beak, from the tip to the anterior angle of the nostril, gave values of 4.6 ± 0.1 mm (mean $\pm$ s.e.m.) for six control embryos, corresponding to stage 41–42 of Hamburger and Hamilton¹⁷, and 3.5 ± 0.1 mm for six embryos treated with 10 mg MUX; the difference is highly significant ($P < 0.001$ by *t*-test). The limbs of treated embryos are shorter and plumper than those of control embryos (Fig. 1*a*), in keeping with a generalised impairment of growth. The difference in growth rate persists and is even accentuated in embryos approaching term. Careful examination of several control embryos and embryos treated with MUX on day 9, which were subsequently fixed and stained for calcified tissue¹⁸ on day 16–21, failed to reveal any gross difference in skeletal morphology or ossification pattern other than an overall reduction in size. This is shown in Fig. 1*d*, which compares the skeletons of a newly hatched (21-d) control chick and a surviving 23-d-old embryo that had been injected with 10 mg MUX on day 9. Thus the xylosides do not apparently cause skeletal distortions, but decrease the overall growth rate of cartilage and bone.

When they are removed from the egg, treated embryos show spontaneous movement of the wings, legs and beaks. These are less vigorous and extensive than the movements of control embryos, and they subside more rapidly. To test whether these differences reflect a general weakness of connective tissue, we measured the tensile strength of the necks of 16-d-old control and MUX-treated embryos immediately after they were killed. For eight control embryos, the mean tension ($\pm$ s.e.m.) necessary to part the neck within 1 min was 384 ± 18 g, while for seven treated embryos only 250 ± 17 g was required. The difference is highly significant, suggesting that treatment with xylosides leads to general weakening of muscles and connective tissues.

The changes that we have described have been produced consistently by injection of 10 mg MUX or 5 mg NPX into 9-d-old embryos (Table 1). Lower doses of xylosides gave rise to embryos that were either outwardly normal or showed the same abnormalities; only a small fraction of the embryos that received a lower dose had clearly recognisable intermediate abnormalities. Table 1 also shows that only the xylosides produced the morphological abnormalities; equivalent doses of the β -glucosides or β -galactosides, or of the aglycones in combination with xylose, had no effect. Essentially the same spectrum of abnormalities, comprising oedema of the soft tissues and interference with growth, has been produced by injecting β -xylosides into embryos at any age between 7 and 12 d.

Table 1 Incidence of morphological abnormalities after treatment with β -xylosides and related compounds

Expt no.	Treatment of embryos	No. of embryos			
		Injected	Dead	No obvious abnormalities	Abnormal
1	MUX (10 mg)	28	11	0	17
	MUX (5 mg)	10	2	1	7
	4-Methylumbelliferone (6 mg)	23	6	19	0
	p-Xylose (4 mg)	23	9	16	0
	4-Methylumbelliferone (6 mg) + xylose (4 mg)	9	1	8	0
	4-Methylumbelliferone- β -glucoside (10 mg)	10	2	8	0
	None (corn oil)	29	8	20	1
2	NPX (5 mg)	24	15	0	9
	NPX (2.5 mg)	14	11	1	2
	p-Nitrophenyl- β -glucoside (10 mg)	14	2	12	0
	p-Nitrophenyl- β -galactoside (10 mg)	14	0	14	0
	None (saline)	10	0	10	0

Derivatives of 4-methylumbelliferone were injected as a suspension in 0.1 ml corn oil; derivatives of *p*-nitrophenol were dissolved in 0.3 ml 0.15 M NaCl. Eggs were injected on day 9 and examined on day 16.

Table 2 Chondroitin lyase digests of glycosaminoglycans from MUX-treated and control embryos

Treatment of embryos	No. of embryos	Mean ($\pm$ s.e.m.) % of liberated disaccharides as		
		6-Sulphate	4-Sulphate	Unsulphated
None (corn oil)	10	44.2 $\pm$ 1.9	35.5 $\pm$ 2.2	20.2 $\pm$ 1.7
MUX (10 mg)	9	21.7 $\pm$ 1.8	17.7 $\pm$ 1.9	60.6 $\pm$ 2.9

Eggs were injected on day 9; 16-d-old embryos were homogenised with 2 vol water, using a Brinkman Polytron operated at full speed. Portions of the homogenate (5 ml) were made 0.005 M in EDTA, 0.005 M in cysteine, 0.1 M in phosphate buffer, pH 6.5, and 1 mg ml⁻¹ in papain (Worthington; 2 $\times$ crystallised), and incubated for 24 h at 57°C. Proteins were precipitated by addition of 0.05 vol of 6.1 M trichloroacetic acid; after clarification by centrifugation, the supernatants were dialysed against four changes of water and freeze-dried. The residues were dissolved in 0.2 ml water; 0.05 ml of 'enriched Tris buffer'²² was added, followed by 0.1 unit of chondroitin lyase ABC (EC 4.2.2.4; Miles) and the mixtures were incubated for 16–20 h at 37°C. The incubation mixtures were applied to Whatman No. 1 paper with the appropriate disaccharide markers²², and chromatographed in *n*-butanol/acetic acid/1 M NH₃ (2:3:1 by vol) for 40 h. Sections of the chromatograms corresponding to the markers were eluted with 1.5 ml 0.01 M HCl and assayed using the periodate–thiobarbiturate reaction²³.

Preliminary chemical analysis of 16-d-old treated embryos indicated a slight increase in wet weight and decrease in dry weight as compared to control embryos. The fraction of body water in nine treated embryos was 90.8 $\pm$ 0.5%, significantly higher than the value of 85.1 $\pm$ 0.5% for nine control embryos ($P < 0.001$ by *t*-test). The amount of DNA per embryo was 15–20% lower in 12 treated embryos than in 12 control embryos; this indicates that the xylosides

interfere slightly with cell division over the period studied, during which the weight of the embryo normally increases by a factor of 10 (ref. 19). The ratio of total protein/DNA was 10–15% lower in the treated than in the control embryos. Determinations of total hydroxyproline showed a similar trend; however, the ratio of uronic acid/DNA was approximately 30% higher in the treated embryos. Details of these and other analyses will be published elsewhere.

A major difference was seen when the chain lengths of the glycosaminoglycans released by treating homogenates of whole embryos with alkali were examined by gel filtration (Fig. 2). There was a marked shift of the elution pattern of glycosaminoglycans from treated embryos towards higher K_{av} values, indicating a 15–20% decrease in average molecular weight. The elution patterns shown in Fig. 2 were reproduced almost exactly with extracts of three embryos treated with 10 mg MUX and four control embryos. Figure 2 also shows that the glycosaminoglycans from MUX-treated embryos had some 4-methylumbelliferone associated with them, suggesting the presence of methylumbelliferyl-chondroitin sulphate. A further marked difference between treated and control embryos was in the proportions of 6-sulphated, 4-sulphated and unsulphated disaccharides released by chondroitin lyase ABC (EC 4.2.2.4) digestion of their total glycosaminoglycans (Table 2). The degree of sulphation of chondroitin sulphate plus dermatan sulphate, calculated from the data in Table 2, was 80% for control embryos and 40% for the treated embryos. These changes in chain length and degree of sulphation are qualitatively similar to those seen in short-term incubations of embryonic cartilage *in vitro*^{15,16}.

Drugs other than MUX (NK Laboratories) and NPX (Pierce) were from Sigma.

KENNETH D. GIBSON
HERBERT J. DOLLER

Department of Physiological Chemistry
and Pharmacology,
Roche Institute of Molecular Biology,
Nutley, New Jersey 07110

RICHARD M. HOAR

Department of Toxicology,
Hoffmann-La Roche Inc.,
Nutley, New Jersey 07110

Received 19 December 1977; accepted 7 March 1978.

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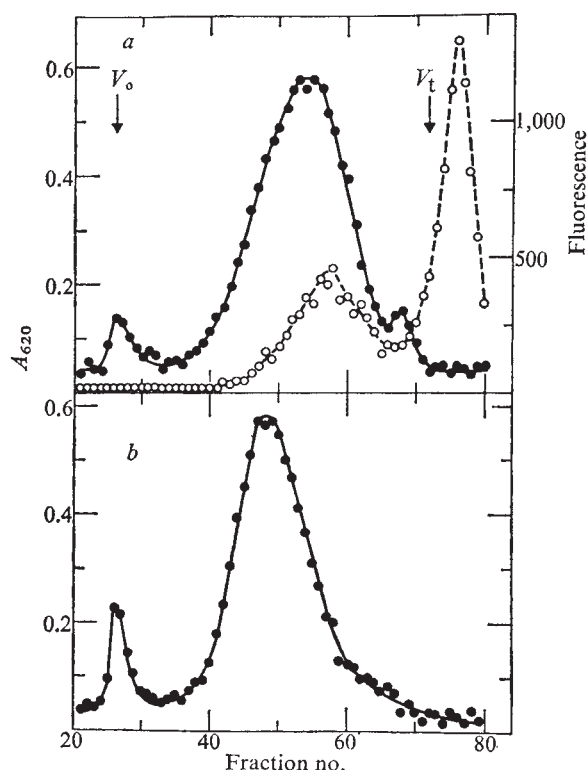


Fig. 2 Gel filtration of glycosaminoglycans; 16-d-old embryos were homogenised with 2 vol water, using a Brinkman Polytron operated at full speed. Portions (2 ml) of the homogenates were mixed with 0.5 ml 0.5 M KOH and kept at 20°C for 24 h. 1 M Na acetate, pH 5.8 (0.2 ml) and glacial acetic acid (15 μ l) were added, and the mixture was clarified by centrifugation. 1 ml of the supernatant solution was applied to a column (1.2 $\times$ 97 cm) of Sepharose 6B (Pharmacia) at 4°C, and eluted with 0.2 M NaCl at a flow rate of 6 ml h⁻¹. Fractions of 1.40 ml were collected. Portions (0.5 ml) of each fraction were analysed for glycosaminoglycan content by mixing with 5 ml 0.05% Alcian blue in 0.05 M MgCl₂–0.05 M Na acetate, pH 5.8, washing the precipitate with 1 ml 95% ethanol, dissolving it in 1 ml 10% Na dodecyl sulphate and determining the optical density at 620 nm (ref. 20). Fractions were also analysed for their content of methylumbelliferone by fluorimetry after acid hydrolysis²¹; the fluorescence is expressed in arbitrary units. *a*, Elution pattern of glycosaminoglycans (●) and methylumbelliferone (○) from a 16-d-old embryo treated with 10 mg MUX on day 9. *b*, Elution pattern of glycosaminoglycans from a control embryo. V_0 and V_t are the void volume and total volume of the column, respectively.

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Suppressor T cells in experimental autoimmune haemolytic anaemia

IMMUNISATION of mice with cross-reacting rat erythrocytes results in the development of autoimmune haemolytic anaemia, as indicated by haematological studies and erythrocyte autoantibodies^{1,2}. However, the disease is self-limiting in normal mouse strains, and we present here evidence for the activity of a suppressor T cell which specifically controls the autoantibody component of the response, while leaving the heteroantibody component unaffected.

The regime we used for induction of anti-erythrocyte autoantibody was four weekly intraperitoneal injections of 2×10^8 red blood cells (RBC) from Wistar rats, and the mouse strains used in these experiments were CBA (Ca) and (NZB $\times$ BALB/c)F₁ hybrids^{1,3}. Anti-erythrocyte antibody was detected by a direct Coombs test (DCT) using a polyvalent rabbit antiserum to mouse immunoglobulin. A semiquantitative scoring system was used, based on the degree of agglutination (see legend to Fig. 1). Rat agglutinins were measured directly in microtitre plates using doubling dilutions of heat-inactivated mouse sera and freshly washed rat RBC at 2% in 1% bovine serum albumin in phosphate-buffered saline.

The first indication that a suppressor cell might be functioning in this system came from our observations in the CBA mice that a secondary challenge with the same regime of four rat RBC injections resulted in an accelerated induction of Coombs positivity in the recipients, followed by an accelerated disappearance of autoantibody. The positive DCT was seen to diminish even while the rat injections were in progress, a situation markedly different from that seen in the primary response to this course of injections (Fig. 1a). In analogous experiments in the (NZB $\times$ BALB/c)F₁ hybrid (which spontaneously develops a positive DCT in later life)⁴, this fall in DCT during the secondary challenge did not occur (Fig. 1b).

The possibility that this accelerated decline might be attributable to a suppressor cell and not due to homeostatic effects of antibody itself was tested in cell transfer experiments. Spleen cells (80×10^6) from those animals which had received two courses of rat RBC were injected intravenously into normal recipients 18 h before the first of four rat RBC injections. This transfer of 'primed' spleen cells resulted in

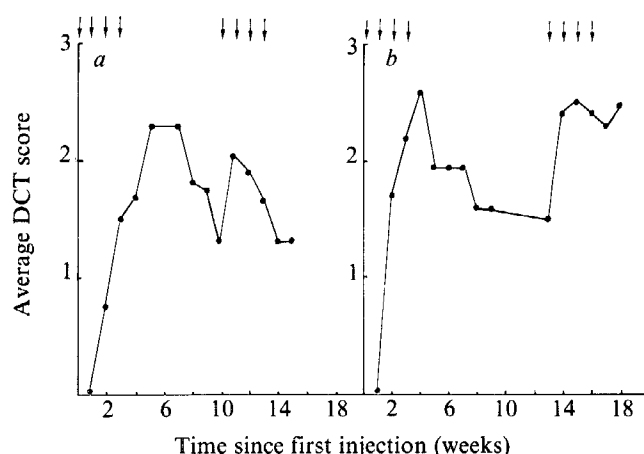


Fig. 1 Positive direct Coombs test (DCT) results in a, CBA; and b, (NZB $\times$ BALB/c)F₁ hybrid mice. Arrows denote intraperitoneal injections of 2×10^8 rat RBC. The DCT scores are the average of groups of 10 mice, the individual samples being allocated points as follows: negative, 0; agglutination visible only under the microscope, 1; agglutination visible with the naked eye, 2; massive agglutination involving all the cells, 3.

significant suppression of the induction of autoantibody in the recipients (Fig. 2). Normal spleen cells exerted no such suppression.

The specificity of this suppression was tested by examining the effect of these 'suppressor' cells on the rat agglutinin response of the recipients, and also on a primary response to sheep RBC, an unrelated antigen. It was found that the splenic suppressor cells affected only the autoantibody response, the response to rat RBC and to sheep RBC being wholly unaffected (Table 1).

We next determined the conditions required for the generation of suppressor cells. It proved unnecessary to carry out two courses of rat RBC injections to generate suppressor activity, as significant suppression was found reproducibly in the spleens of animals following only two injections of rat RBC. In some, but not all, experiments, suppression was observed with mouse spleens following only one injection of the antigen; most of these mice never became DCT positive. Thus, suppressor cells can apparently develop in the absence of detectable autoantibody. The suppressor cell is long-lived, activity being still present in the spleens of animals six months after their last injection (Fig. 2).

To identify the type of cell causing the suppression, we repeated the transfer experiments using separated cell populations. Spleen and lymph node cells from rat RBC-primed and control (non-primed) animals were separated twice on nylon wool columns by modification of the method of Julius *et al.*⁵. Cells passing through such columns have

Table 1 Specificity of suppression

Cells injected	RBC†	Spleen cells‡	DCT* score	Agglutination titre*		Anti-sheep RBC* (PFC per spleen)	
				Rat RBC	Sheep RBC	Direct	Indirect
Rat $\times$ 4	—	—	2.1	11 $\pm$ 1.6	—	—	—
Rat $\times$ 4	Normal	—	2.0	9 $\pm$ 2	—	—	—
Rat $\times$ 4	Suppressor	—	0.5	11 $\pm$ 0.9	—	—	—
Sheep $\times$ 4	Normal	—	0	—	10 $\pm$ 2	12,600 $\pm$ 8,800	674,000 $\pm$ 5,200
Sheep $\times$ 4	Suppressor	—	0	—	10 $\pm$ 2	7,000 $\pm$ 220	652,000 $\pm$ 6,400

Agglutination titre is expressed as the last well giving agglutination, using doubling dilutions of the test sera and starting the dilutions at $\frac{1}{2}$.

*Four weeks after the first injection of RBC, 5 CBA mice per experimental group.

† 2×10^8 RBC intraperitoneally.

‡ 100×10^6 cells intravenously.

been shown to be predominantly T cells. Some of the adherent cells were recovered from the nylon wool by agitation and squeezing in cold minimum essential medium (MEM). The cells, after separation, were washed and injected intravenously into the recipients. Immunoglobulin staining of the separated cell populations with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse Fab showed these procedures to have enriched the populations for T or B cells, respectively, as shown in Table 2. It was found that substantial depletion of B cells from the suppressor cell populations had no effect on the suppressor activity. 25×10^6 or even 15×10^6 T-enriched cells were just as effective as 50×10^6 unseparated cells in suppressing the induction of autoantibody in the recipients. The B-cell enriched population (25×10^6), on the other hand, contained no suppressor activity (Table 2). Using the Wilcoxon ranking test, the differences in DCT score between the animals receiving suppressor T cells and the control animals are significant for $2\alpha=0.01$. There is no significant difference between the animals receiving B cells and the control populations. This experiment clearly indicates that the suppressor cell in the spleens and lymph nodes of rat RBC-primed mice is neither an adherent cell nor a B cell, and we conclude that it is probably a T cell. Similar results have been obtained by D. Naysmith (personal communication).

It could be argued that the failure to suppress the rat agglutinin response is more apparent than real, owing to the antagonistic and compensating effects of B-memory (positive) and T-suppressor (negative) cells for the anti-rat RBC response. However, the separated T cells did not suppress the anti-rat RBC agglutinin response (Table 2). Furthermore, the separated B cells apparently did not transfer an increased response to rat RBC. Alternatively, one might argue that increased T-cell memory for rat RBC conceals suppression. This would be unlikely, as it is believed that the induction of autoimmunity in this system is due to cross-reaction between rat and mouse at the T-helper cell level; that is, the same helper T cells are involved in both¹. Moreover, the rat RBC agglutinin response is

Table 2 Suppressor activity of different lymphoid populations before and after separation on nylon wool columns

Donor cell population	%Ig staining	DCT score* in recipients	Rat agg.* titre in recipients
Unseparated suppressors (spleen+lymph node)	40	0.8	12 ± 0.4
Suppressor B-depleted ('T')	11	0.5	12 ± 1
Control B-depleted ('T')	9	1.6	10 ± 1
Suppressor 'B'-enriched	87	2	12 ± 0.8
Control (spleen+lymph node)	44	1.7	—

* Mice tested 4 weeks after the first injection of RBC.

relatively independent of T-helper cells (comparison between athymic *nu/nu* and littermate mice, unpublished data).

A third possibility is that the suppressor cell is specific, not for antigen but for a particular antibody class or subclass, so that an autoantibody that can circulate on the red cells (positive DCT) is replaced by one that causes red cell destruction (negative DCT). Class switches have been shown to occur in this system⁶, but the DCT remained positive throughout, unlike the situation reported here.

The results demonstrate the presence of a suppressor cell whose activity is specifically directed against autoantibody production, and indicate that this cell is a T cell. An essentially similar suppression, probably mediated by T cells, has been shown to occur during the induction of allergic encephalomyelitis in rats⁷. Allison *et al.* have suggested that a suppressor T cell may play a crucial part in controlling the emergence of autoaggressive clones of B cells, and have proposed that deficiency of such a cell may play a role in the genesis of autoimmune disease. De Heer and Edgington⁸ have evidence to support the concept that autoimmune responses result from the derepression of multiple clones of B lymphocytes and that the autoimmune haemolytic anaemia of the NZB stems from an immunoregulatory defect. We are currently investigating whether the hyper-responsiveness of the NZB and the (NZB $\times$ BALB/c)F₁ hybrid to the induction of autoantibody³ is due to a loss of specific suppressor cells, as suggested by the failure of the hybrid to regulate its secondary DCT response (Fig. 1), or whether all suppressor cells are deficient in these strains, as suggested by experiments in which suppression is nonspecifically induced by concanavalin A (ref. 10).

How the T cells achieve such a specific suppressive effect is unknown, but one obvious possibility is that they are directed against idiotypic constituents of the autoantibody and/or of the helper T cell; immunoregulatory models of this kind are currently attracting widespread interest¹¹.

A. COOKE

P. R. HUTCHINGS

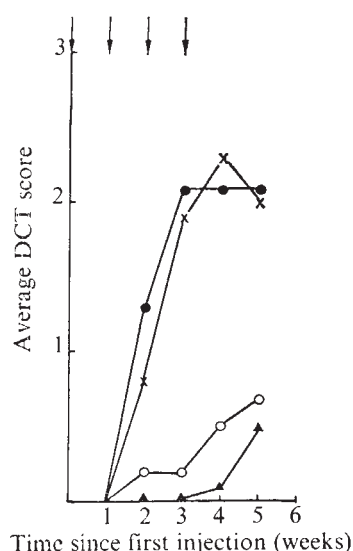
J. H. L. PLAYFAIR

Department of Immunology,
Middlesex Hospital Medical School,
London, UK

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Fig. 2 Positive direct Coombs test (DCT) results in CBA mice following 0.4 ml intravenous injections containing: ●, 80×10^6 normal mouse spleen cells; ○, 75×10^6 spleen cells from mice 6 months following one course of 4 weekly rat RBC injections; ▲, 80×10^6 spleen cells from mice 3 weeks following a second course of 4 weekly rat RBC injections; X, untreated mice. All mice received the first 4 weekly injections of 2×10^6 rat RBC 18 h later. DCT scores as in Fig. 1.



Temperature-induced seizure and frequency-dependent neuromuscular block in a *ts* mutant of *Drosophila*

SINGLE-GENE conditional mutants of *Drosophila melanogaster* which affect both development and the physiology of the adult present a valuable opportunity for studying the effects of a specific biological lesion on both individual physiological processes, and on whole animal biology encompassing the growth and development of the organism. Temperature-sensitive mutations at the *shibire* locus of *D. melanogaster* produce both developmental and neurophysiological defects during various periods of the animal's life cycle, and thus illustrates how a single gene product can participate in both synaptic transmission in the adult as well as in the process of differentiation during development. The importance of analysing such a mutant lies in the hope of revealing a biological mechanism common to both adult physiology and the developmental process. Such a revelation might contribute insights into both processes. The work presented here is an analysis of the adult physiological phenotype of the *shibire* mutant.

Temperature-sensitive mutants at the *shibire* locus were first isolated in the laboratory of David Suzuki¹. The mutants have been termed temperature-sensitive paralytics, although the findings presented here of an extensive and prolonged temperature-induced outbreak of neuronal activity suggest that they might, more appropriately, be termed temperature-sensitive epileptics. The *shi^{ts1}* strain exhibits a host of developmental defects when shifted to temperatures above 29 °C for short periods during development². In the adults there occurs spontaneous muscle firing, loss of the transients in the electroretinogram (ERG)³, and the reported failure of neuromuscular transmission at high temperature^{4,5}. Ikeda *et al.* have also reported⁴ that, at the non-permissive temperature, the muscle membrane is still electrically excitable, the muscle resting potential is still normal, and nerves retain their capacity to transmit impulses. The findings presented here are that a sudden and extensive increase in neuronal electrical activity accompanies the shift to the non-permissive temperature and is prolonged while the animal is maintained at that temperature, that extensive electrical activity at the neuromuscular junction always precedes a block in neuromuscular transmission; and that a decline in neuromuscular transmission does not occur at the non-permissive temperature if spontaneous neuronal activity is blocked and the neuromuscular junction is stimulated at a frequency less than 0.6 Hz.

A hypothesis to account for the biological lesion of the *shibire* mutation must therefore take into account the excitatory effects within the ganglia and inhibitory effects on neuromuscular transmission. The following observations and experiments suggest that a temperature-induced depolarisation of pre- and postsynaptic neuronal membranes might account for the physiology of the adult.

For normal growth and development flies were grown at 22 °C. The *shibire* allele used in all of these studies was the *shi^{ts1}* allele. For heating and cooling during experiments, the fly was mounted by fixing the legs and abdomen in wax on to a brass-covered chamber through which ethylene glycol at the appropriate temperature was circulated. The time necessary to increase the temperature from 27 to 31 °C was 3 min. The preparation was protected from air currents and the temperature was constantly monitored by a thermistor at the side of the fly.

Tungsten microelectrodes were used for extracellular recording of nerves at the roots of the thoracic ganglion. For short flash ERGs a metal microelectrode was inserted into the eye. A light source for ERGs was pro-

vided by a General Radio Strobotac 1531-AB strobe light.

Stimulation of the dorsal roots of the ventral ganglion was accomplished by inserting, at an angle of 45° to the horizontal, two metal microelectrodes (separated by approximately 100 µm), into the anepisternum, rostral to the pleural wing process and dorsal to the pleural cleft, in order to intercept the root of the second dorsal nerve. Recordings were made from dorsal lateral indirect flight muscles (DLM) fibres ipsilateral to the point of insertion, and delay between stimulus and response was 0.80 ± 0.15 ms at 26 °C.

For intracellular recording from the DLM, it was found that results obtained using tungsten electrodes insulated with lacquer to within 1.0 µm of the tip (Frederick Haer, & Co.) gave essentially the same results as KCl-filled micro-pipettes (a.c. recording only). These tungsten electrodes were used because they penetrated the cuticle without previous preparation of the animal. For experiments requiring perfusion, flies were mounted as detailed above. A micro-pipette was inserted into the abdomen and the perfusion fluid was pumped through the body cavity. The composition of the physiological saline used has been described previously⁶. Substances were added to the perfusion fluid in concentrations stated in the text. Control wild-type flies were perfused in the same manner both with and without the added substances.

Figure 1 summarises the physiology of the *shi^{ts1}* mutant during a temperature shift experiment. Both the outbreak of neuronal activity and the depression of neuromuscular transmission are illustrated.

The temperature-induced burst of neuronal activity was found to occur at all the major roots of the thoracic ganglion as well as the cervical connective. The increase in activity at these points was monitored by extracellular recording. A representative record from the third ventral root that supplies the metathoracic leg is shown in Fig. 1d.

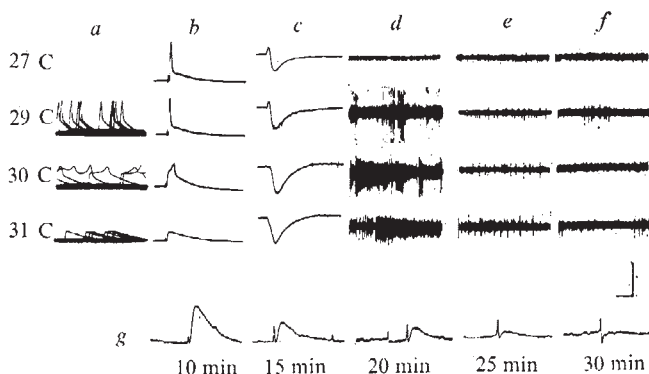


Fig. 1 Summary of *shi^{ts1}* physiology during a temperature shift experiment. *a*, Intracellular recording of spontaneous activity from DLM fibre. Muscle responses are occurring at a frequency of 3–6 Hz; *b*, artificially evoked activity recorded from DLM fibre produced by electrical stimulation of motor nerve (responses in *a* and *b* were recorded almost concurrently from the same electrode). *c*, Concurrent loss of transient from short flash ERG (rate of stimulation = 1 Hz). *d*, Extracellular record of activity recorded from root of third ventral nerve. *e*, *shi^{ts1}* fly treated with 1 mg ml⁻¹ phenobarbital, electrode position as in *d*. *f*, Wild-type control, electrode position as in *d* and *e*. In *d*, *e* and *f* heads were removed from preparations to minimise phasic activity (*d*, *e* and *f* are from a.c. recordings). *g*, Continuing spontaneous activity in DLM fibre over the time period indicated. E.j.ps occurred at a frequency of 3–6 Hz throughout the time period. Time and voltage marks: *a* and *b* 10 ms, 40 mV; *c* 40 ms, 8 mV; *d*, *e* and *f* 0.4 s, 1 mV; *g* (at 10 min, 10 ms, 4 mV; at 15, 20 and 25 min, 10 ms, 1 mV; at 30 min, 10 ms, 0.5 mV).

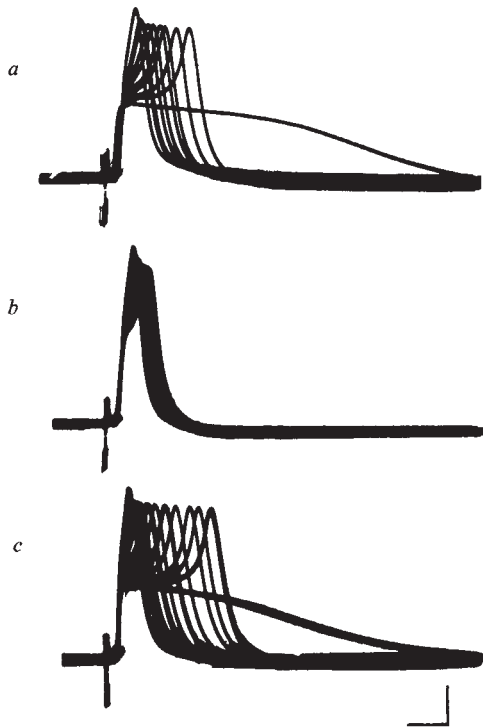


Fig. 2 Responses of *shi^{ts1}* DLM fibre to electrical stimulation of motor nerve at 32°C after treatment with 1 mg ml⁻¹ phenobarbital before temperature shift. Spontaneous activity did not occur in this fibre during the temperature increase: *a*, initiation of stimulation at 4 Hz; *b*, subsequent stimulation of the same fibre at 0.5 Hz after a resting interval of 5 s; *c*, initiation of stimulation at 4 Hz after *b*. Time and voltage mark: 5 ms, 10 mV.

Activity at the neuromuscular junction during the shift to the non-permissive temperature was monitored by intracellular recording from DLM fibres. In the instance illustrated in Fig. 1*a*, spontaneous electrical activity continued in this fibre until 30 min after the temperature shift. The frequency of firing was approximately 5 Hz. During this time the electrically excitable component of the muscle response was lost followed by the slow decline of the excitatory junction potentials (e.j.ps), until only a small spike that preceded each e.j.p. remained (Fig. 1*g*). Extracellular recording from the dorsal roots of the thoracic ganglion that supply the DLM fibres, also showed continuing spontaneous neuronal activity during and after this time period. Electrical stimulation of a motor nerve leading to a muscle fibre undergoing spontaneous activity, evoked muscle responses with shapes characteristic of the spontaneous activity occurring in the fibre (Fig. 1*b*).

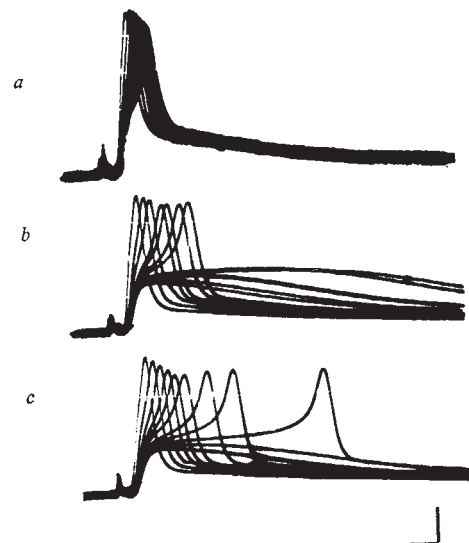
A frequency-dependent decline in neuromuscular transmission was revealed at the non-permissive temperature by blocking all spontaneous temperature-induced activity with barbiturate drugs (Fig. 1*e*), and testing the neuromuscular junction for failure at different frequencies of stimulation. At the non-permissive temperature, both barbiturate-treated animals and untreated wild-type control animals could tolerate a stimulus frequency of 25 Hz without a decline in neuromuscular transmission. Barbiturate-treated *shi^{ts1}* flies, however, showed a decline in neuromuscular transmission only if stimulated at a frequency greater than 0.5 Hz at the non-permissive temperature (Fig. 2). The effect of temperature on the *shibire* neuromuscular junction is to lower the tolerance of the neuromuscular junction to high frequency stimulation. A similar frequency-dependent decline in neuromuscular transmission was found in *shi^{ts1}* flies not treated with barbiturate (at a temperature just

below the point of spontaneous firing) (Fig. 3). This latter observation indicates that the frequency-dependent failure of transmission in the drug-treated *shibire* flies is not an anomalous result of the interaction of barbiturate with the *shibire* lesion.

A similar frequency-dependent loss of the transient in the ERG was noted in barbiturate-treated *shi^{ts1}* flies. In these experiments barbiturate-treated *shi^{ts1}* flies showed no loss of the transients at the non-permissive temperature if stimulated at a frequency below 0.6 Hz. However, stimulation at higher frequencies produced a decline in the response. (The ERG transients may represent the response of second order neurones in the visual pathway^{7,8}.)

The interpretation of these phenomena has been difficult. However, it is proposed that the depression of neuromuscular transmission might be explained by a temperature-induced vulnerability of the presynaptic terminal to depolarisation, that is enhanced by electrical activity at the neuromuscular junction. Such depolarisation might result in presynaptic inhibition⁹⁻¹¹, and/or a depletion of neurotransmitter from presynaptic terminals¹². In fact, indirect evidence supporting a theory of depolarised nerve terminals in *shibire* flies shifted to the non-permissive temperature, has come from ultrastructure studies of the *shibire* neuromuscular junction. It has been found that shifting *shibire* flies to the non-permissive temperature for a period of 30 min produces a depletion of normal synaptic vesicles from the presynaptic terminal and an accumulation of recycled vesicle membrane in the form of large cisternae. (It is also notable that this condition can be avoided by previous treatment of flies with barbiturate, C. Poodry and R. Edgar, unpublished.) Such a condition (in *Rana pipiens*) has also been reported to be produced by intentional depolarisation of the neuromuscular junction with high levels of extracellular potassium¹². (It can, however, also be produced by prolonged high frequency stimulation¹³, but this might

Fig. 3 Intracellular recordings from *shi^{ts1}* DLM fibre: responses to electrical stimulation of motor nerve at temperatures just below the outbreak of spontaneous activity. *a*, Initial responses at 27°C to stimulation at 20 Hz; *b*, initial responses at 28°C to stimulation at 10 Hz; *c*, initial responses at 28.5°C to stimulation at 6 Hz. The maximum rates of stimulation which did not produce decremental responses at these temperatures were approximately: 27°C=20 Hz; 28°C=7 Hz; 28.5°C=4 Hz. In each case the electrically excitable response returned when the fibre was rested for 5 s. If stimulation was continued after loss of the electrically excitable component, the amplitude of the e.j.ps declined. (Note that preparation has not been treated with barbiturate in this experiment.) Time and voltage mark: 2 ms, 10 mV.



also work by way of synaptic depolarisation¹¹.) Whether the presynaptic terminals of some interneurons are similarly effected is unknown, but the possibility cannot be ruled out.

Discussion of the above explanation with respect to spontaneous neuronal activity is complicated by the fact that the source of this neuronal activity is, at present, unknown. However, one possibility is that the *shibire* lesion effects postsynaptic neuronal membranes in a manner similar to the presynaptic effect, thus causing a depolarisation. The effect on the postsynaptic cell would be excitatory^{9,14} and certain cells within the ganglion, having low thresholds, might be brought to their firing levels. However, the existence of heterogeneity in the synaptic membranes of different neurones is not an unlikely possibility. Thus, some synapses may be invulnerable to the *shibire* lesion, whereas others are effected in a variety of ways. It should also be mentioned that ERG analysis of *shibire* mosaics indicated both a pre- and postsynaptic site of action of the *shibire* lesion³.

In conclusion then, it can be stated that, although the exact nature of the effect remains elusive, it is likely to involve a defect in both pre- and postsynaptic membranes of neurones. With regard to the developmental defects, it is not inconceivable that the same membrane defect could also be present in embryonic cells, which, when subjected to elevated temperatures, produced the developmental defects observed in *shibire* flies. Investigation of the role of membrane depolarisation and conductance in this regard is the subject of further investigation.

We thank David Bentley, Richard Steinhardt and Regis Kelly for discussions.

This work was supported in part by a Biomedical Support Grant (NIR-S05-PR7006-10) to L.K. L.S. is a recipient of a NIH Predoctoral Training Grant (5 T32 GMO7127).

LAWRENCE SALKOFF
LEONARD KELLY

Department of Genetics,
University of California,
Berkeley, California 94720

Received 8 September 1977; accepted 7 March 1978.

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Soma potential of an interneurone controls transmitter release in a monosynaptic pathway in *Aplysia*

In the molluscan nervous system, action potentials and synaptic potentials invade the soma so that its potential reflects intrinsically and extrinsically initiated electrical activity¹. However, its functions related to the electrical activity of the neurone are unknown. Recently, it was shown that the soma potentials of arthropod interneurons controlled transmitter release on to motoneurons^{2,3}. In *Aplysia*, soma potentials of some interneurons have been observed to have an effect on the size of post synaptic potentials (p.s.ps) in the cell with which they synapse; the possibility that p.s.ps were affected by changes in temporal

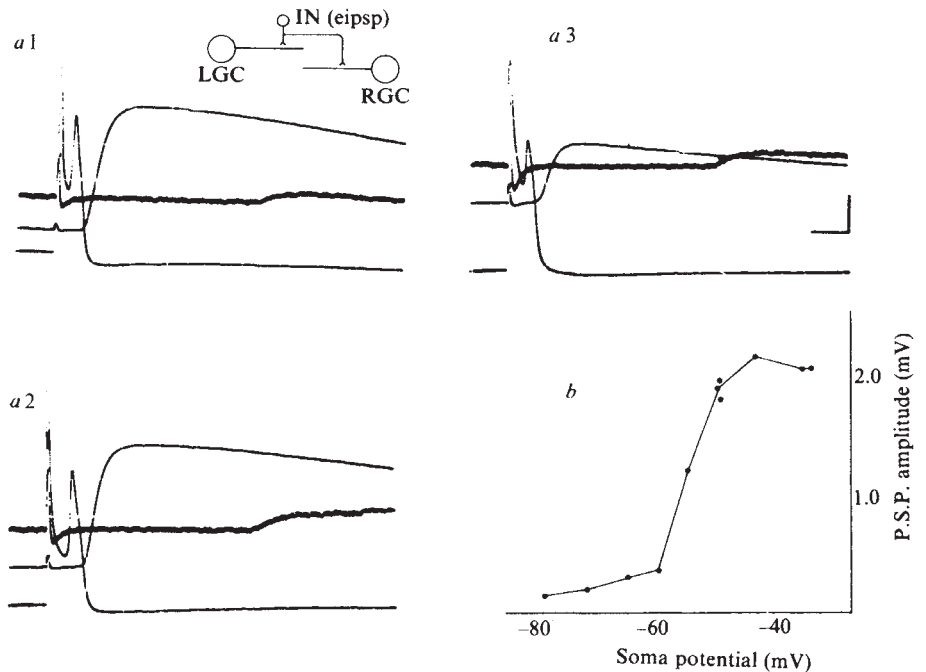
properties of the spike induced by the soma potential⁴ or electronic coupling between cells⁵ was not excluded. We investigated this observation and report here that soma potentials of one interneurone regulate the release of transmitter, possibly by a direct effect on its terminals. Release can be sustained beyond the normal time course of the p.s.p. by prolonged depolarisation of the soma.

Experiments were carried out in *Aplysia californica* using the isolated left pleural ganglion in which the left giant cell (LGC) and identifiable interneurons synapsing with it are located⁶. One such interneurone (IN) evokes p.s.p. consisting of a rapid excitatory phase followed by a longer lasting inhibitory phase (termed e.i.p.s.p.)⁴. This pathway is consistent with criteria ascribed to monosynaptic pathways⁴. The connective tissue overlying the ganglion was removed by micro-dissection to facilitate visualisation and impalement of the neurones. The ganglion was pinned out in a small lucite chamber, and the preparation was continuously perfused with artificial seawater⁷ kept at 16 °C. Double-barrelled microelectrodes filled with 3M KCl were used to impale the LGC and the IN. During the experiments the soma potential of the LGC was held at its resting level of about -55 mV by passing current through one barrel of the electrode. The p.s.p. amplitude evoked in LGC was a measure of transmitter release.

In 16 experiments we found that spiking of IN, with its soma hyperpolarised, always resulted in decreased p.s.p. amplitudes in LGC as compared to those evoked at resting levels, usually 40-45 mV (Fig. 1a, b). (We primarily investigated the excitatory phase of the p.s.p., although both it and the inhibitory phase were clearly affected.) The largest decrements were seen when soma potentials were set between -45 and -60 mV. Beyond -60 mV, the decrements were more gradual; in some preparations in which IN was hyperpolarised to -120 mV p.s.ps were still evoked by a spike initiated in the soma. Depolarisation of the soma resulted in an increase of p.s.p. amplitude. Above -35 mV, depolarisation resulted in little increase, as if maximum release had been attained. (Release can be increased further at resting and depolarised potentials by increased external Ca²⁺, 33 mM.) Figure 1a shows that the size of the soma spikes at the three different levels of polarisation are about the same, yet the p.s.p. is smaller with soma hyperpolarisation; in some preparations, spikes were larger when the soma was hyperpolarised. The temporal properties of the spikes seemed to be the same. Attempts to relate p.s.p. amplitude to spike size, as in the squid giant synapse^{6,7} were unsuccessful in that the correlation was poor; relationship between the size of the p.s.p. to spike overshoot was also poor. However, a consistent relationship existed between p.s.p. amplitude and soma potential; the relation is seen as an S-shaped curve (Fig. 1b), which corresponds to the input-output curves in the squid giant synapse⁸⁻⁹.

In an attempt to exclude the possibility that changes of soma potential altered temporal properties of the spikes or blocked conduction along axonal branches, we simultaneously recorded p.s.ps in the right giant cell (RGC), in the abdominal ganglion, and in LGC evoked by intracellular stimulation of IN; each is a monosynaptic pathway⁴. The estimated distances between the soma of IN and its synaptic contacts with RGC and LGC are several centimetres and 200 μ m, respectively. Figure 1a shows that the size of p.s.ps in RGC are unaffected by IN hyperpolarisation. If partial conduction block had occurred during hyperpolarisation, spike amplitudes must have exceeded the threshold of the IN axon, otherwise p.s.ps would not have been evoked. Synaptic latencies at both LGC and RGC were unchanged as IN soma potential was varied (see legend to Fig. 1). Although these results do not exclude the possibility that soma polarisation alters axon spikes, they do suggest that polarisation has an effect on terminals in close proximity to the soma,

Fig. 1 Interneurone soma potential regulation of p.s.p. amplitude. *a*, See inset; simultaneous recordings from right giant cell, RGC (upper trace) and left giant cell, LGC (middle trace) to IN stimulation (lower trace); IN soma potential set at (1) 35.3, (2) 44.1, and (3) 66.6 mV; 44.1 mV was resting level. 1–2 ms pulses were applied to interneurone within 5 s of setting soma potentials; stimuli were applied at 1 min intervals to avoid decremental effects of repeated activation of the two monosynaptic pathways. Synaptic latency in LGC was 7 ms, average for this synapse, and 90 ms in RGC. *b*, p.s.p. amplitude in LGC as function of IN soma potential, obtained from a different preparation from that of *a*. Latencies at 36.7, 50.2, 60.4 and 78.7 mV are 7, 7, 7 and 7.5 ms, respectively. Maximum effect of soma potential on p.s.p. occurs between resting level, 50 mV here, and 60 mV in all experiments; the regulation constant, voltage input-output ratio, was approximately 0.2 for results both in *a* and *b*. Scale, IN, 20 mV; LGC, 2 mV; RGC, 0.5 mV, 20 ms.



because it had an effect on LGC p.s.ps and not on those of RGC.

For the results as typified by Fig. 1, soma potentials were set within 5 s before IN was stimulated. Possibly, conditioning was necessary for that length of time in order to affect conductances at the terminal membrane, somewhat analogous to the potentiating effect of tetanic stimulation for several seconds. Figure 2 shows that the effect of soma depolarisation on transmitter release occurs within 350 ms, and thus, that longer term conditioning was not necessary. Other observations show that the minimum time for release to be affected is about 50 ms. This range of latencies is well within the duration of synaptic inputs to IN⁹.

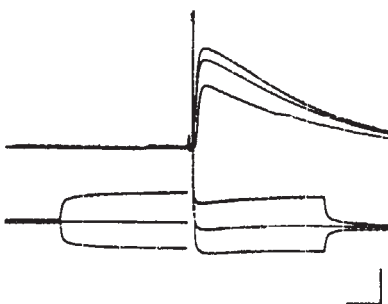
The results of the key experiments are shown in Fig. 3. We attempted to evoke transmitter release by depolarising the soma in the absence of a Na-spike. The ganglion was perfused with 94 μ M (ref. 10) of tetrodotoxin (TTX). It is noteworthy that release occurred and was sustained during a 2-s depolarisation pulse (Fig. 3a). Ordinarily, the p.s.p. duration is about 500 ms (ref. 4). When depolarisation was increased progressively, the size of the p.s.p. also increased, and release continued throughout 2-s long pulses (Fig. 3a, b). After termination of the pulse, the p.s.p. showed a slow decline; this is reminiscent of the after-effects of injecting Ca into the presynaptic terminal of the squid giant

synapse¹¹. Sustained transmitter release was also seen in Na-free seawater (not shown). The onset of depolarisation elicited an apparent Ca-spike in the soma⁵ to which the initiation of release was related. Antidromic stimulation of an IN axon failed to elicit a soma-spike, showing that the spike is nonconducting with TTX present. In an attempt to initiate release without eliciting spikes, we slowly increased depolarisation so that the soma membrane accommodated to the stimulating current. In the absence of a spike, we were able to detect small p.s.ps when the soma was depolarised to 55 mV above the resting potential⁹. Depolarisation of LGC to -10 mV resulted in a decrease of the p.s.p. (Fig. 3c), indicating that the equilibrium potential was being approached, a characteristic associated with chemical synapses. Hyperpolarisation of IN had no effect on LGC, which excludes the possibility that the two neurones were electrotonically coupled. These findings are more direct evidence that soma polarisation controls release at the terminals.

The evidence that IN soma polarisation affects the terminal region is indirect, and does not exclude possible effects of a polarisation on axon-spikes. We have no reliable measure of axon-spike amplitude, as both the stimulating and recording electrodes were in the soma. The soma is not in the conduction pathway between the trigger zone and its synaptic input, for they are closer to each other than to the soma^{1,12}. A similar relationship may exist between the terminals and trigger zone, but with the terminals electrically close to the soma, because hyperpolarisation decreased p.s.p. size and did not block the spike. With the soma electrically close to its terminals, electrotonic potentials can mediate soma polarisation at the terminals, analogous to that reported in arthropod neurones^{2,3}. If soma hyperpolarisation brought larger spikes in the terminals, p.s.p. size should have increased as in the squid giant synapses^{6,7}. IN soma spikes were almost the same size as those at resting levels, yet the p.s.ps were smaller (Fig. 1a).

Hyperpolarising pulses of less than 50 ms, which is sufficient time to alter spike amplitudes, had no measurable effect on p.s.p. size. This tends to exclude the possibility that altered spikes are sufficient to explain our results. Apparently, soma polarisation of at least 50 ms is necessary to condition the terminals before spike invasion. The best supporting evidence for our proposal is with TTX and Na-free seawater; a Na-spike is unnecessary to initiate release,

Fig. 2 The effect of short duration polarisation of IN soma on p.s.p. Upper trace, LGC, largest p.s.p. to 16 mV depolarisation, next at resting level, and smallest to 16 mV hyperpolarisation of soma. Spike initiated 350 ms after onset of polarisation. Scale, LGC 5 mV; IN 20 mV; 0.1 s.



and there is little evidence to suggest that Ca-spikes are regenerative. Consequently, spikes invading the terminals are not a prerequisite for the liberation of transmitter^{7,8} (Fig. 3). Further, continuous release with long depolarising pulses is more easily explained by soma polarisation affecting the release mechanism at the terminals than by altered axon-spikes. This direct relationship of release to soma depolarisation would be analogous to the relationship of release to depolarisation of the pre-fibre in the giant synapse^{7,8}. Hyperpolarisation may partially suppress the release mechanism, possibly by altering Ca-flux, which is a potential-dependent flux in the giant synapse^{7,8} and in the squid axon^{13,14}. If this is so, it would help to explain our findings in terms of those from the giant synapse.

These experiments showed that soma potentials regulate phasic release of transmitter. We also found that soma polarisation influences post-tetanic potentiation in a similar manner, that is, depolarisation enhances it⁹. Consequently, synaptic plasticity can also be similarly controlled. As the IN LGC synapse is one of several found in the pleural ganglion¹, it is apparently useful to indicate how soma potential control of transmission is incorporated into the functions of a neurone such as IN. Changes in soma potentials result from invasion by spikes and after-potentials and by synaptic activity in the isolated and intact nervous system. Another source contributing to the potentials may be the activation of receptors on the soma surface¹⁵ by transmitters. As the soma potential is an integrated ex-

pression of intrinsic and extrinsic potential inputs, it can modulate synaptic transmission even though spikes invade the terminals of the neurone.

We thank Dr L. Tauc for making facilities available to carry out this work and for useful discussions, and Drs R. T. Kado and J. Prichard for critically reading an earlier draft of this paper. This work was partially supported by an INSERM grant, 76.4.053.6 and a grant from La Fondation Medicale Francaise to L. Tauc. T.S. is an Attaché de Recherche to INSERM. B.P. was a recipient of a Josiah Macy Faculty Scholar Award and of an NIMH grant.

T. SHIMAHARA

B. PERETZ*

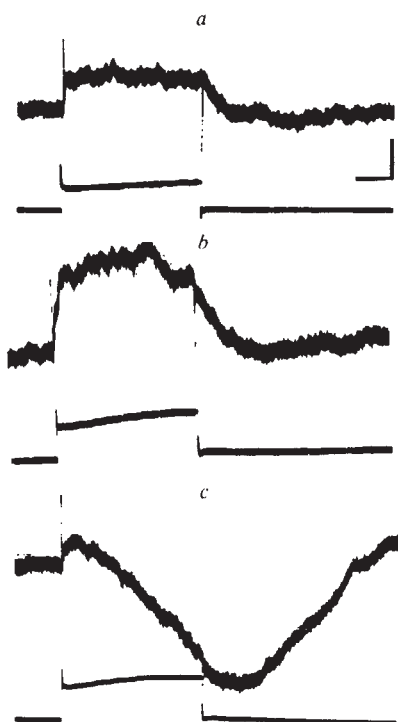
Laboratoire de Neurobiologie,
Cellulaire du C.N.R.S.,
91190 Gif sur Yvette, France

Received 14 November 1977; accepted 6 March 1978.

*To whom reprint requests should be addressed at the Department of Physiology and Biophysics, University of Kentucky Medical Centre, Lexington, Kentucky 40506.

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Fig. 3 Sustained transmitter release during IN soma depolarisation after 45 min perfusion with TTX. *a*, The soma was depolarised 56 mV, which results in a spike, p.s.p. in LGC 0.2 mV. *b*, depolarisation was increased to 100 mV, p.s.p. increased to 0.4 mV; a gradual increase of release occurs as the IN soma continues to depolarise. Release continued throughout 2 s of depolarisation. *c*, Effect of LGC depolarisation on sustained release. IN soma was depolarised to the same level as in *b*; the excitatory phase of the p.s.p. is decreased, as LGC was depolarised near the equilibrium potential; the inhibitory phase is now clearly expressed, as the depolarisation displaces LGC farther away from the equilibrium potential. The inhibitory phase seems to start coincident with or near that of the excitatory phase. Scale: *a*, *b*, *c*, upper (LGC) 0.2 mV; lower (IN) 0.1 V and 0.5 s.



Influence of carbon dioxide on level of ionised calcium in squid axons

SQUID axons contain about 0.1–0.5 mmol calcium per kg axoplasm, of which only about 0.1 μ mol is ionised^{1–4}. Although this level of ionised calcium can be made to change by altering either intracellular binding or membrane transport, little is known about the factors that regulate ionised calcium in physiological conditions. We now report experiments showing that one such factor might be the $p\text{CO}_2$.

Axons were cleaned of adhering small nerve fibres, loaded by microinjection with the calcium-sensitive photoprotein aequorin, mounted in a simple flow cell, and the light output monitored as described previously². Axons were superfused with artificial seawater containing (mM): NaCl, 432.5; MgCl_2 , 100; CaCl_2 , 10; KCl, 10; and NaHCO_3 , 2.5 at pH 7.8 when fully equilibrated with air.

When the external pH was varied by addition of 10 mM buffer containing equimolar amounts of PIPES and HEPES, the light emission remained relatively constant over the pH range 6.0–8.0. There was, however, a fall in light output after transfer to a solution in which 32.5 mM of the NaCl had been replaced by NaHCO_3 and the mixture equilibrated with 5% CO_2 /95% O_2 to give a final pH of 7.2. Figure 1 shows that this effect is fully reversible, is observed in both sodium- and lithium-containing seawaters containing calcium, but is not detectable in the absence of extracellular calcium. In some experiments, the initial decrease in light output in the presence of CO_2 was followed by a maintained increase that recovered on return to normal seawater.

As CO_2 readily permeates cells, acidifying the intracellular environment^{5–7}, the most likely explanation for these observations is that they are brought about by a decrease in intracellular pH perhaps acting directly on the aequorin^{8,9}. Our own measurements and those of Boron and de Weer⁶ show that exposure to seawater equilibrated with 5% CO_2 lowers the intracellular pH to about 6.7. If this change in

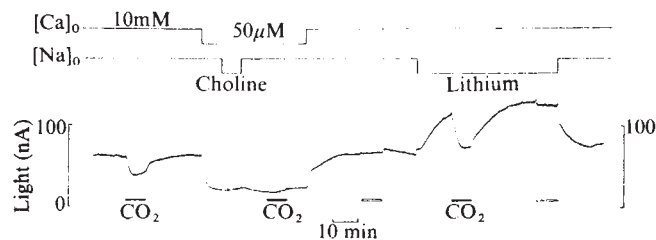


Fig. 1 Light output (nA) from an axon of *Loligo forbesi* injected over 1 cm with aequorin. The method and composition of artificial seawater are described in the text. Changes in composition of the superfusing solutions are indicated above the light trace. For the periods indicated, 400 mM sodium was replaced by an equimolar amount of choline or lithium. Solid bars indicate periods of exposure to seawater containing 35 mM NaHCO₃ and equilibrated with 5% CO₂/95% O₂ and the open bars periods of exposure to seawater containing 10 mM NH₄Cl. Temperature, 20 °C. Axon diameter, 800 μm.

pH is acting directly on the aequorin, it is difficult to understand why the light output is decreased by CO₂ in calcium-containing media but not in calcium-free ones, and why it is biphasic in some preparations. It seemed essential to calibrate the aequorin reaction directly. An arrangement geometrically similar to that of an axon was used. A solution of aequorin was sealed inside a porous cellulose acetate tube 800 μm in diameter¹⁰, which was mounted in the flow system in the same way as an axon. A stable rate of light emission was obtained for many hours in the presence of a low level of ionised calcium (0.5 M KCl, 20 mM potassium phosphate pH 7.2, 5 mM EGTA and 2 mM CaCl₂). Because of the sensitivity of Ca-EGTA buffers to pH, it was desirable to obtain a stable light output in a medium lacking a calcium buffer. This was achieved by making use of the fact that the aequorin reaction is strongly inhibited by magnesium ions. A stable light output—comparable to that found in axons—was obtained in a medium containing 400 mM MgCl₂, 350 μM CaCl₂ and 10 mM K-HEPES at pH 7.2. The immediate response to an increase in pH (to 8.0) was a decrease in light output, and to a decrease in pH (to 6.5 or 6.0) was an increase; but in the conditions of our assay the aequorin was not stable at acid pH. At constant pH, CO₂ had no obvious effects of its own.

Our tentative conclusion is that the fall in light output seen in axons exposed to CO₂ is unlikely to result from a direct effect on the aequorin reaction and presumably reflects a genuine decrease in ionised calcium inside the axon. The secondary increase in light output seen in some axons may result from a direct effect on the aequorin or an increase in intracellular ionised calcium. Figure 1 also includes two exposures to NH₄Cl which should have alkalised the interior of the axon⁶. Neither exposure produced a marked change in light emission but in some experiments an increase was observed. It may be that for the experiment illustrated in Fig. 1 no change in light output reflects an actual increase in ionised calcium because at alkaline pH the aequorin reaction rate is depressed.

The mechanism by which CO₂ brings about a decrease in ionised calcium could involve intracellular binding or membrane transport. The second possibility seems more likely, because acidification of isolated axoplasm containing aequorin only elicits an increase in light output, and direct measurement of calcium transport has revealed that it is sensitive to intracellular pH. Baker and McNaughton¹¹ have shown that a reduction in the intracellular pH of squid axons—either by injection of a pH buffer or by exposure to an increased pCO₂—inhibits calcium-dependent sodium efflux^{12–14} and we have now shown by

direct measurement that the associated influx of ⁴⁵Ca is also inhibited. In addition, ⁴⁵Ca efflux tends to be reduced but to a lesser extent. These data suggest that the decrease in intracellular calcium brought about by an increase in pCO₂ is mediated through a fall in intracellular pH inhibiting mainly internal sodium-dependent calcium influx. We have also carried out some experiments in which the interior of an axon was made alkaline by inclusion of 10 mM NH₄Cl in the seawater. In these conditions both ⁴⁵Ca influx and the associated external calcium-dependent sodium efflux are stimulated.

These observations on squid axons could have wide physiological implications. Sodium-calcium exchange is particularly noticeable in muscle as well as nerve¹, and it would, for instance, be of interest to know whether the reduction in contractility of both cardiac and smooth muscles brought about by an elevated pCO₂ also involves alterations in membrane calcium transport. Should it prove to be a general finding that the calcium influx system in nerve and muscle is sensitive to internal pH, this might have profound implications for the regulation of ionised calcium in extracellular fluid because conditions that alter intracellular pH could result in large shifts of calcium between extracellular fluid and cells.

P.F.B. was supported by grants from the MRC and the Wellcome Trust and P.H. was in receipt of a grant from the Deutsche Forschungsgemeinschaft. We thank Dr O. Shimomura for a gift of purified aequorin.

P. F. BAKER

P. HONERJÄGER

Department of Physiology,
King's College,
Strand, London WC2, UK and
Laboratory of the Marine Biological Association,
Plymouth, UK

Received 11 February; accepted 30 March 1978.

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Calcium-dependent release of somatostatin and neurotensin from rat brain *in vitro*

THE peptides somatostatin and neurotensin were first described in extracts of mammalian hypothalamus^{1–3}. The recent development of sensitive radioimmunoassay techniques and the application of immunohistochemical studies have shown that these peptides are concentrated in nerve terminals in various regions of the central nervous system (CNS)^{4–12}. Somatostatin-containing nerve terminals are particularly abundant in the median eminence of the hypothalamus, from which somatostatin seems to be released as a hypophysiotropic hormone controlling the secretion of growth hormone from the anterior pituitary⁷. Somatostatin

is also present in a variety of glandular tissues, in the gastrointestinal tract^{8,9}, and in nerve terminals in many areas of the CNS outside the hypothalamus⁴⁻⁶. Neurotensin is similarly present in high concentration in the hypothalamus, and is also found in other areas of the CNS, in the gastrointestinal tract and in pituitary gland¹⁰⁻¹². Within the CNS, the unique localisation of these peptides in specific systems of neurones suggests that they may be released as neurotransmitters or neuromodulators, as has been proposed for other neuropeptides such as substance P (ref. 13) and the enkephalins¹⁴. So far, however, it has not been shown that somatostatin or neurotensin can be released from CNS neurones, although a calcium-dependent release of somatostatin was recently reported from neurohypophyseal tissue *in vitro*¹⁵. We describe here the release of both somatostatin and neurotensin from rat brain tissue *in vitro* by a calcium-dependent mechanism, thus lending further support to the hypothesis that they may normally be released from nerve terminals within the CNS.

Hypothalamic tissue was dissected from chilled rat brain, and the tissue from one brain, weighing approximately 40 mg, was cross-cut at 200- μ m intervals using a McIlwain tissue chopper. The resulting slices were suspended in Krebs-bicarbonate medium in a small plastic superfusion chamber at 37 °C, as described previously¹⁶. The slices were superfused at 37 °C with a modified Krebs-bicarbonate solution, containing bovine serum albumin and bacitracin to protect against loss of released peptides by adsorption or

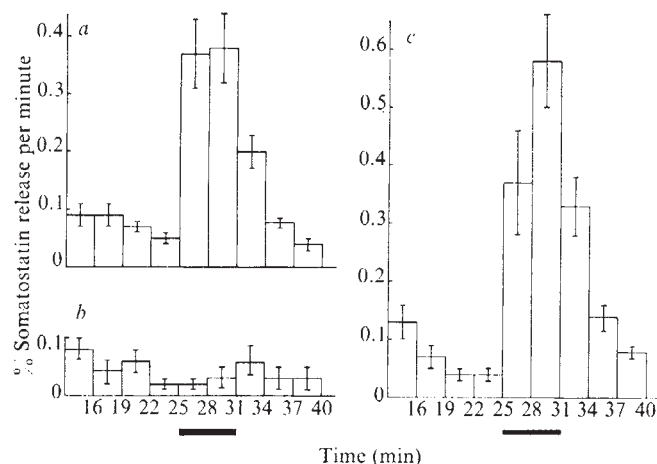


Fig. 1 Potassium-evoked release of somatostatin from rat hypothalamus slices. Slices of rat hypothalamus or amygdala were superfused with a modified Krebs-bicarbonate solution at 37 °C, at a rate of approximately 300 μ l min⁻¹. The medium had the following composition: NaCl 127 mM; KCl 3.83 mM; CaCl₂ 1.8 mM; KH₂PO₄ 1.18 mM; MgSO₄ 1.18 mM; NaHCO₃ 20 mM; D-glucose 2 g l⁻¹; bovine serum albumin (Sigma, crystalline) 0.1%; bacitracin (Sigma) 30 μ g ml⁻¹. The medium was gassed with an oxygen/carbon dioxide (95/5 v/v) mixture. During the period indicated by the horizontal black bars the slices were exposed to a medium in which KCl was substituted for NaCl to give a final K⁺ concentration of 50 mM. Duplicate 50- μ l aliquots of the superfusate samples (collected at 3-min intervals) were analysed for somatostatin content using a radioimmunoassay procedure¹⁷. Results are expressed as percentage total tissue somatostatin released per minute, based on measurements of the tissue content at the end of the superfusion. This was estimated after extraction of the tissue with boiling 1 M acetic acid, homogenisation, centrifugation and freeze-drying of samples of the resulting supernatant for subsequent radioimmunoassay¹⁷. Control values for hypothalamic slices (a) are mean $\pm$ s.e.m. for 12 experiments, and results obtained with calcium-free media in hypothalamic slices, (b) are mean $\pm$ s.e.m. for four experiments. c, Potassium-evoked release of somatostatin from slices of rat amygdala (mean $\pm$ s.e.m. for seven experiments).

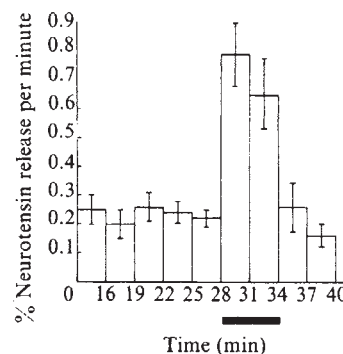


Fig. 2 Potassium-evoked release of neurotensin from slices of rat hypothalamus. Experimental details as in Fig. 1. Duplicate 100- μ l aliquots of superfusate samples were used for neurotensin measurements using a radioimmunoassay method¹⁸. The superfusate samples and tissue extracts were the same as those used for measurements of somatostatin release (Fig. 1), but results are shown only for those experiments in which there were measurable amounts of neurotensin in the resting release samples (mean $\pm$ s.e.m. for six experiments).

metabolism. After an initial wash period of 13 min, superfusate samples were collected at 3-min intervals and aliquots of the samples were assayed for somatostatin and neurotensin using sensitive radioimmunoassay procedures^{17,18}. In some experiments samples of amygdala, including the overlying pyriform cortex, were dissected from left and right sides of chilled sections of rat brain (wet weight of pooled tissue from one rat brain approximately 50 mg), and the tissue was sliced and superfused as described above. At the end of each experiment the hypothalamic or amygdalar tissue was recovered and the content of somatostatin and neurotensin measured, using a radioimmunoassay method. Release results are expressed as fractional rate constants.

After the initial wash period, only very small amounts of somatostatin were detected in the superfusate samples, although the tissue contained relatively large amounts of each peptide (Fig. 1). The content of somatostatin was below the lower limit of accurate measurement using the radioimmunoassay method (approximately 70 pg ml⁻¹) in approximately one-third of such samples, and the basal efflux was equivalent to less than 0.1% of tissue stores per minute. During a 6-min exposure to Krebs solution containing high potassium (50 mM KCl) there was a large increase in the rate of release of somatostatin from both hypothalamic and amygdalar tissue (Fig. 1a and c) amounting to increases of 6- to 10-fold over resting efflux values. The increased release was sustained throughout the duration of the 6-min exposure to high potassium, and was higher in some instances during the second half of this period than initially (Fig. 1c). When calcium was omitted from the superfusion solution, the resting efflux of somatostatin was somewhat reduced and there was no response at all to high potassium (Fig. 1b). Comparison of tissue somatostatin contents at the end of a 40-min superfusion (hypothalamus, 37.0 $\pm$ 3.32 ng; amygdala, 33.3 $\pm$ 2.67 ng) with those measured in freshly dissected tissue (hypothalamus, 36.4 $\pm$ 2.93 ng; amygdala, 37.6 $\pm$ 9.24 ng) showed that there was no significant loss of the tissue stores from either hypothalamus or amygdala during this period. The amount of somatostatin present in the amygdala and adjacent cortex was surprisingly large, being equal to that in the hypothalamic samples. The amount of somatostatin measured in the superfusate samples during the entire period of superfusion amounted to less than 5% of the total tissue stores in each brain region, and the amount released during a 6-min period of exposure to high potassium was equivalent to approximately 2% of the total tissue content.

As described previously⁴, the concentration of neurotensin in hypothalamic tissue (3.6 $\pm$ 0.24 ng; mean $\pm$ s.e.m., n = 8)

was approximately 10 times lower than that of somatostatin. The amounts of neurotensin measured in release samples were often too low to be accurately measured (less than 10 pg ml^{-1}). In those experiments in which neurotensin release could be measured, however, the rate of spontaneous release was equivalent to a loss of approximately 0.25% of total tissue stores per minute, and exposure to high potassium elicited a highly significant three-fold increase in release (Fig. 2). During superfusion with calcium-free media, the release of neurotensin was too low to be measured (results of four experiments), and exposure to high potassium failed to elicit any measurable release of the peptide. The amounts of neurotensin released during superfusion of amygdalar samples were too low to be measured. As with somatostatin, there was no significant reduction in the content of neurotensin in hypothalamic tissue after superfusion for 40 min *in vitro*, compared with values obtained from freshly dissected tissue.

Our results show that both somatostatin and neurotensin can be released from rat hypothalamus by depolarising concentrations of external potassium, and that this release is calcium-dependent. The stability of the tissue stores of the peptides during superfusion, and the relatively small amounts released spontaneously or during potassium stimulation, are similar to the behaviour of putative amine and amino acid CNS transmitters^{19,20}, and the putative peptide transmitter substance P (ref. 16). Our results differ, however, from those we have reported for enkephalin release from rat brain *in vitro*²¹, where a substantial proportion of the tissue stores could be released by high potassium and a major loss of peptide occurred during superfusion. The finding that somatostatin can be released from both hypothalamic and non-hypothalamic tissue supports the hypothesis that it may act as a neurotransmitter or modulator in the CNS, in addition to its probable neuroendocrine functions.

This work was supported by a grant from the William Randolph Hearst Foundation and by grant No. DA-01785.

L. L. IVERSEN*

S. D. IVERSEN†

F. BLOOM

C. DOUGLAS

M. BROWN

W. VALE

Salk Institute,
San Diego, California 92112

Permanent addresses: *MRC Neurochemical Pharmacology Unit, Department of Pharmacology; † Department of Experimental Psychology, University of Cambridge, UK.

Received 28 December 1977; accepted 6 March 1978.

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Immunoreactive α -MSH in human plasma in pregnancy

It has been suggested that the increased skin pigmentation of pregnancy is due to increased secretion of melanocyte-stimulating hormone (MSH) (refs 1–3), but this is not β -MSH, because the slight increase in immunoreactive β -MSH found in late pregnancy⁴ is much less than the changes associated with skin pigmentation. Evidence suggests that the 22 amino acid β -MSH, originally thought to be the major MSH in man⁵, is an extraction artefact⁶, and the immunoreactive β -MSH measured in the human^{4,7,8} is in fact β or γ lipotrophin⁹. Other forms of β -MSH have never been isolated from the adult human pituitary and only small quantities of α -MSH have been found¹⁰. Immunoreactive α -MSH is present in larger amounts in the human foetal pituitary¹¹ and has also been detected in the pituitary of the pregnant woman¹². We report here the presence of circulating immunoreactive α -MSH in pregnancy.

Venous blood was collected between 10.00–12.00 into polystyrene tubes containing EDTA, centrifuged as soon as possible, and the plasma separated and stored at -20°C . Immunoreactive α -MSH was measured by the method of Penny and Thody¹³. Plasma samples were diluted fourfold and assayed at three doubling dilutions in replicates of five. This dilution gave a lower limit of detection of 80 pg ml^{-1} .

Plasma samples were obtained from five groups of subjects: first trimester ($n = 24$), third trimester ($n = 26$), parturition (maternal and cord samples) ($n = 17$), post partum (6 weeks)-lactating ($n = 8$), and post partum (6 weeks)-nonlactating ($n = 18$). Results were compared with those from a control group of 27 patients (13 male, 14 female), aged 16–77 yr, who were outpatients with minor skin disorders, and with plasma samples obtained from six patients with hypopituitarism.

The immunoreactive α -MSH content of three placentae was also measured. The placentae were frozen at -70°C immediately after collection, and when required for assay were thawed, trimmed of cord and membranes, rinsed in ice cold saline and weighed. A portion of each placenta was extracted in 1 N HCl and serial dilutions were assayed for α -MSH.

Immunoreactive α -MSH was detected in the plasma of only one control patient (Fig. 1), and no patients with

Fig. 1 Plasma immunoreactive α -MSH levels in control subjects (a, 27) and during the first (b, 24) and third (c, 26) trimester of normal pregnancy, and at 6 weeks nonlactating (d, 18) and lactating (e, 8) post partum. ND = not detectable ($< 80 \text{ pg ml}^{-1}$).

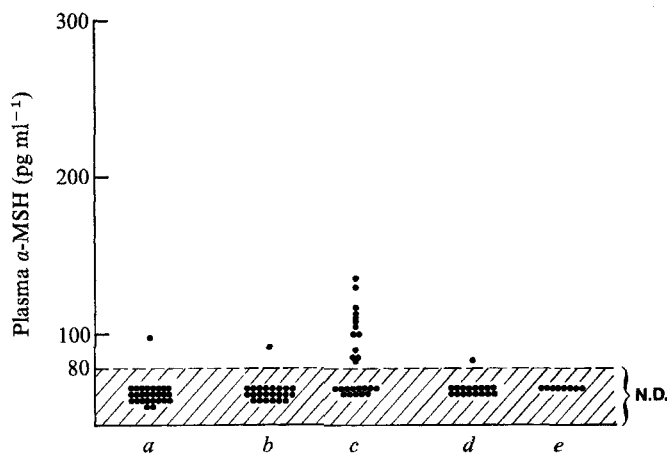


Table 1 Immunoreactive α -MSH in maternal and cord plasma

Patient	Maternal α -MSH (pg ml ⁻¹)	Cord α -MSH (pg ml ⁻¹)
C	<80	80
P	84	80
W	161	80
H	84	80
G	81	87
S	147	107
Wi	<80	<80
Wa	166	130
M	197	123
B	113	<80
Go	<80	<80
Ga	<80	<80
F	<80	<80
Mi	95	<80
A	<80	112
Ch	<80	<80
Bi	<80	<80

hypopituitarism. Similarly, immunoreactive α -MSH was undetectable in plasma of most subjects during the first trimester (Fig. 1). However, one subject had 269 pg ml⁻¹, the highest we have so far encountered in the human (Fig. 1). During the third trimester, 50% of subjects had detectable levels of plasma immunoreactive α -MSH, with a mean $\pm$ s.e.m. of 108 ± 5 pg ml⁻¹ (Fig. 1). At parturition, immunoreactive α -MSH was found in the plasma of nine subjects (mean $\pm$ s.e.m. 125 ± 14 pg ml⁻¹) and in five of the cord samples (mean $\pm$ s.e.m. 119 ± 7 pg ml⁻¹) (Table 1). No immunoreactive α -MSH was found in the plasma of women who were breast feeding and in only two women of the nonlactating group (Fig. 1). The immunoreactive α -MSH content in the three placentae ranged over 1.33–2.07 ng per g wet weight (mean $\pm$ s.e.m. 1.76 ± 0.22 ng per g) (Table 2).

Table 2 Immunoreactive α -MSH in human placentae

Weight of placenta (g)	α -MSH Total (μ g)	(ng per g wet weight)
1	500	0.95
2	550	1.15
3	650	0.85
	567 ± 44	0.98 ± 0.09
		1.76 ± 0.22

Although the present assay shows a high specificity for α -MSH, and the displacement curves produced by the plasma samples paralleled those of synthetic α -MSH, the immunoreactivity could be due to the larger peptide characterised by Lee and Lee¹⁴. Alternatively, in late pregnancy substances may be present in plasma which cause nonspecific reactions in our radioimmunoassay.

Only small amounts of α -MSH occur in the adult human pituitary¹⁰, which would explain our failure to detect circulating α -MSH in control subjects. The high levels of α -MSH in late pregnancy are remarkable and should be confirmed using extraction methods. A source of these raised α -MSH levels might be the foetal pituitary, although it is unlikely that α -MSH can cross the placental barrier. Although the maternal pituitary may produce α -MSH during pregnancy¹², extrapituitary sites such as the placenta, which produces ACTH¹⁵, must be considered as a source of α -MSH in pregnancy.

It seems unlikely that α -MSH has a pigmentary role in pregnancy because the pigmentation begins early and is normally focal, unlike that of Addison's and Cushing's syndrome. A sebotrophic role^{16,17} is similarly unlikely because

the seborrhoea of pregnancy persists during lactation¹⁸, when α -MSH was not elevated. α -MSH from the foetal pituitary may, however, stimulate production of the vernix that protects foetal skin from maceration by the liquor. This may have longer-lasting consequences, as adult rats show increased sebum secretion after pre- and neonatal administration of α -MSH^{19,20}. α -MSH also stimulates the foetal adrenal cortex, and the switch to an ACTH control may be of significance in parturition^{11,21}. In addition, α -MSH stimulates foetal growth in the rat and may have a similar function in man²². Finally, because of its actions on the CNS^{23–25}, the presence of α -MSH during early life could be of considerable importance for brain development.

We thank the MRC for financial support (A.J.T. and S.S.).

D. CLARK

A. J. THODY

SAM SHUSTER

University Department of Dermatology,
Newcastle upon Tyne, UK

H. BOWERS

Princess Mary Maternity Hospital,
Great North Road,
Newcastle upon Tyne, UK

Received 28 December 1977; accepted 15 March 1978.

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Pharmacological classification of adrenergic α receptors in the guinea pig

THE actions of sympathomimetic amines have attracted considerable attention for some time. Ahlquist^{1–3} introduced the concept of adrenergic α and β receptors to explain the differences in the effects of some of these amines on various organ systems and then Lands *et al.*^{4,5} suggested the existence of two types of β receptors, β_1 and β_2 . It has been claimed that there is a pharmacological difference between α receptors at adrenergic neurones and those in smooth muscle^{6–9}. I have previously shown that the inhibitory α receptor of the cholinergic neurone in guinea pig ileum is pharmacologically different from the excitatory α receptor located in the smooth muscle of the guinea

pig ileocaecal sphincter and rabbit aorta¹⁰⁻¹³. Langer¹⁴ suggested that there are two kinds of α receptors, α_1 and α_2 (see also ref. 15). Adopting this nomenclature, it was proposed that α_1 receptors are present when the relative affinity of phenylephrine for the receptors is stronger than that of clonidine, and α_2 receptors when the relative affinities are reversed¹³. α_1 Receptors have thus been shown to be present in smooth muscle cells of rabbit aorta and pulmonary artery, and in guinea pig ileocaecal sphincter; and inhibitory α_2 receptors in cholinergic neurones of guinea pig ileum and in adrenergic neurones of rabbit pulmonary artery and rat vas deferens. I report here the results of further studies supporting the theory of two types of α receptors.

Several further agonists showing α -stimulatory properties were tested on isolated guinea pig aorta¹⁶ and electrically stimulated guinea pig ileum¹⁷. Twenty hours before being killed, the animals were injected with 5 mg reserpine (Serpasil) per kg body weight intraperitoneally (i.p) to prevent interference from endogenous release of catecholamines¹⁸. The organs were mounted in Krebs solution in a water-jacketed organ bath at 37 °C. The solution contained sotalol 5×10^{-5} M to block β receptors¹² and cocaine 3×10^{-5} M and corticosterone 3×10^{-5} M to block neuronal and extraneuronal uptake of catecholamines⁷. When guinea pig ileum was being tested, the Krebs solution contained 2×10^{-5} M choline in order to 'feed' the cholinergic nerves and preserve their acetylcholine stores¹⁹. Electrical stimulation of the ileum at 0.1 Hz, with 1-ms pulses, induced twitch contractions that were mediated by stimulation of cholinergic neurones, as shown by their abolition by atropine or tetrodotoxin¹³. In innervated ileum, the α receptors were found only at cholinergic neurones as these receptors were absent in the denervated preparation¹².

Dose-response curves for 19 agonists (Fig. 1) were obtained and the results were treated statistically as described in the legend of Table 1. The relative affinity and intrinsic activity (calculated as described in the Table legend, using noradrenaline as reference) were taken as rough measures for the true affinity and efficacy of the tested drug. The pre-post ratio (see legend to Table 1) was taken as a measure of the selectivity for pre- or postjunctional α receptors. The drug with the most selective effect on the presynaptic α adrenoceptors was clonidine (2a), followed by xylazine (3), tramazoline (2b), cyclopentamine (4n), nordephrin (4e), naphazoline (1b) and oxymetazoline (1a). Xylazine showed maximal intrinsic activity at the prejunctional α receptor, whereas it had only a partial agonistic effect at the postjunctional α receptor. The drugs with the most selective effect on the postjunctional α receptor were methoxamine (4m) and phenylephrine (4a). Phenylephrine was more selective than was indicated by the pre-post ratio, as it showed submaximal intrinsic activity in the ileum but not in the aorta. The difference in the pre-post ratio between the extremes, clonidine and methoxamine was 13,000, thus strongly supporting the theory of a difference between the types of receptors studied. These results were similar to those obtained following inhibition of contractions in the electrically stimulated rat vas deferens¹⁵. They were also very similar to those obtained in rabbit pulmonary artery when pre- and postjunctional α receptors were compared. Thus, tramazoline, nordephrin, clonidine, oxymetazoline and naphazoline were preferentially active pre-synaptically, and methoxamine had a preferentially postsynaptic action^{7,8}.

The data indicated that substitution of a phenylethylamine with a methyl group at the α -carbon increased the selectivity for α_2 receptors. This was true in all three cases studied, that is, drug 4a $\rightarrow$ 4j, 4g $\rightarrow$ 4l and 4d $\rightarrow$ 4k. Loss of a 4-hydroxy group at the phenyl moiety, that is, 4g $\rightarrow$ 4d, 4h $\rightarrow$ 4e and 4l $\rightarrow$ 4k, resulted in an increased selectivity for α_1 receptors. This change in the molecule gave a marked loss of both intrinsic activity and relative affinity at the α_2 receptors. Loss of a 3-hydroxy group, that is 4l $\rightarrow$ 4j, 4k $\rightarrow$ 4i and 4h $\rightarrow$ 4c, resulted in some increase in the selectivity for α_2 receptors, although this was not true for 4g $\rightarrow$ 4b.

All imidazoline derivatives (1a, 1b, 2a, 2b) and xylazine (3) showed a preference for α_2 receptors. This was most marked for the drugs in which the phenyl group was substituted only at the 2,6 position (1a and 3).

Recent evidence has shown that α receptors located at sites other than peripheral neurones or smooth muscle cells might belong to either subclass of receptors. The α receptors inhibiting melanin granule dispersion induced by melanocyte-stimulating hormone in frog skin and that inhibiting renin release from rat kidneys might be of the α_2 type. Further, α receptors in the central nervous system which are involved in lowering blood pressure might belong to this group²⁰.

In conclusion, the present results have yielded substantial evidence in support of a subdivision of adrenergic α receptors into α_1 and α_2 types. The order of relative affinities of agonists

Fig. 1 Structural formulae of the tested drugs: oxymetazoline (1a), naphazoline (1b), clonidine (2a), tramazoline (2b), xylazine (3), tyramine (4a), octopamine (4b), oxedrine (4c), noradrenaline (4d), 1-phenylephrine (4e), ethylephrine (4f), 1-noradrenaline (4g), 1-adrenaline (4h), phenylpropanolamine (4i), hydroxy-amphetamine (4j), 1-nordephrin (4k), metaraminol (4l), methoxamine (4m) and cyclopentamine (4n).

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4k	OH	H	OH	CH ₃	H																																																																											
4l	OH	OH	OH	CH ₃	H																																																																											
4m		<table><tr><td>CH₃</td><td>H</td></tr></table>	CH ₃	H																																																																												
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4n		<table><tr><td>H</td><td>CH₃</td><td>CH₃</td></tr></table>	H	CH ₃	CH ₃																																																																											
H	CH ₃	CH ₃																																																																														

Table 1

Drug	n	Guinea pig ileum			n	Guinea pig aorta			Pre-post
		pD_2	Intrinsic activity	Relative affinity		pD_2	Intrinsic activity	Relative affinity	
1a	8	6.95(6.71–7.19)	0.92(0.85–0.98)	1.7	6	6.24(6.01–6.47)	0.96(0.72–1.20)	0.20	8.5
1b	8	6.93(6.67–7.18)	0.24(0.16–0.32)	2.2	6	6.53(6.24–6.82)	0.72(0.66–0.78)	0.20	11
2a	6	7.70(7.48–7.92)	0.52(0.44–0.61)	9.1	6	5.43(4.97–5.90)	0.61(0.50–0.71)	0.016	570
2b	8	7.21(7.00–7.42)	0.40(0.32–0.49)	2.9	8	5.77(5.30–6.24)	0.84(0.68–0.99)	0.054	54
3	8	6.31(6.10–6.52)	1.00(0.91–1.08)	0.50	8	4.76(4.38–5.15)	0.43(0.30–0.56)	0.0035	140
4a	4	< 2.52	—	< 0.000095	6	3.91(3.76–4.07)	0.43(0.34–0.52)	0.00069	< 0.14
4b	7	4.82(4.64–4.99)	0.26(0.15–0.37)	0.011	6	4.50(4.25–4.74)	0.83(0.73–0.94)	0.0027	4.1
4c	7	4.48(4.20–4.77)	0.42(0.33–0.52)	0.0049	5	4.81(4.52–5.10)	0.85(0.73–0.96)	0.048	0.10
4d	6	4.54(4.22–4.87)	0.24(0.14–0.34)	0.0077	8	5.78(5.53–6.03)	0.93(0.80–1.06)	0.063	0.12
4e	6	4.69(4.22–5.16)	0.29(0.24–0.33)	0.0089	6	6.32(6.01–6.63)	0.95(0.90–1.00)	0.12	0.074
4f	6	4.45(3.13–5.77)	0.47(0.24–0.70)	0.0061	8	5.14(4.91–5.37)	0.86(0.66–1.06)	0.0079	0.77
4g	27	6.69(6.58–6.80)	1	1	41	7.09(7.00–7.18)	1	1	1
4h	6	7.19(6.91–7.48)	0.99(0.93–1.05)	3.4	8	7.31(7.20–7.41)	0.95(0.80–1.11)	1.2	2.8
4i	7	3.17(2.99–3.35)	1.07(1.03–1.11)	0.00042	5	4.00(3.78–4.22)	0.72(0.60–0.83)	0.0042	0.1
4j	6	4.37(4.15–4.59)	0.80(0.62–0.98)	0.0063	6	4.19(4.11–4.26)	0.60(0.55–0.65)	0.00086	7.3
4k	7	5.15(4.86–5.45)	0.33(0.21–0.45)	0.023	8	5.38(5.05–5.70)	0.98(0.90–1.05)	0.024	0.96
4l	6	7.25(6.72–7.77)	1.02(0.96–1.08)	3.2	6	6.60(6.33–6.87)	1.11(1.03–1.18)	0.23	14
4m	6	3.39(3.29–3.48)	1.08(0.98–1.19)	0.00044	6	5.25(5.13–5.38)	1.03(0.96–1.10)	0.010	0.044
4n	8	4.02(3.69–4.36)	0.97(0.86–1.08)	0.0029	6	3.42(2.82–4.02)	0.97(0.91–1.03)	0.00014	21

Cumulative dose-response curves for the drugs tested were obtained on coaxially stimulated guinea pig ileum and on guinea pig aorta. The data were fitted to the logistic function: $E = MA^p / (A^p + K^p)$ by means of nonlinear least-square regression, in a computer²¹. In the formula, A is the drug concentration, K the dose at a semi-maximal effect (ED_{50}) and M the maximal effect. P is a constant dependent on the slope of the dose-response curve. pD_2 was given by $pD_2 = -\log_{10}(K)$. The relative affinity was calculated as K_D/K_N , where K_D is the K of the drug and K_N the K of noradrenaline in the same specimen. The intrinsic activity was calculated similarly as M_N/M_D . The pre-post ratio is given as relative affinity in ileum ÷ relative affinity in aorta. n , Number of observations. The values in brackets are the 95% confidence intervals.

may be used as a tool for classification of α receptors. When the relative affinities of phenylephrine and noradrenaline are greater than those of tramazoline, xylazine and clonidine, α_1 receptors are present, and when these orders are reversed, the receptors are of the α_2 type. Methoxamine can also be used for such classification if the ratios of the relative affinities of α_1 and α_2 are calculated instead.

I thank Associate Professor Rolf G. G. Andersson for valuable criticism. This work was supported by grants from the Swedish Medical Research Council (04X-04498).

After submission of this paper, similar findings to those reported here, were published in abstract form²², concerning the nature of the α receptor of the cholinergic neurone in guinea pig ileum.

JARL E. S. WIKBERG

Department of Pharmacology,
Linköping University,
Linköping, Sweden

Direct evidence of two types of β adrenoceptor binding site in lung tissue

THERE is much evidence to suggest that β adrenoceptors are not homogeneous—pharmacological data on the actions of β adrenoceptor agonists and antagonists in intact tissues indicate the presence of two broad subclasses β_1 β_2 (refs 1, 2), although there is some controversy regarding this strict subdivision^{3,4}. The receptors found in heart and adipose tissue have been classified as β_1 , whereas those in liver, skeletal muscle, trachea and uterus have been designated β_2 . This classification has been supported by biochemical studies examining β adrenoceptor-mediated cyclic AMP formation in various tissues^{5–7}, although the characterisation of the β adrenoceptor in lung tissue using this approach has been the subject of some debate^{8,9}. The direct binding of radiolabelled β -adrenoceptor antagonists to tissue membrane preparations has been studied by several workers in an attempt to overcome some of the problems that may arise when examining this receptor indirectly⁹. We have examined the direct binding of the specific β adrenoceptor ligand ³H-dihydroalprenolol (DHA) to rat lung membranes and here we present evidence for the presence of both β_1 and β_2 adrenoceptor subtypes in this tissue in a ratio of approximately 1:3.

The characteristics of the binding of ³H-DHA to rat lung membranes have been described in detail elsewhere¹⁰. Briefly the specific binding (binding that can be displaced by 200 μ M isoprenaline) represents 90% of the total binding, is of high affinity, reversible, stereospecific and has pharmacological characteristics that indicate that the binding is to the β adrenoceptor. (In all figures and tables binding refers to specific binding only.) We have examined the ability of β antagonists to displace ³H-DHA from these membranes. Some of the antagonists used have been reported to possess similar affinities for β_1 and β_2 receptors (non-selective) and others demonstrate a much higher affinity for β_1 receptors.

IC_{50} values (concentrations of agents causing 50% displacement of specific binding) were determined for several

Received 29 November 1977; accepted 24 February 1978.

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of these β antagonists from detailed dose-response curves using eight to 10 concentrations of the test compound. The ability of timolol (a nonselective β antagonist¹¹) to inhibit the binding of ^3H -DHA to lung membranes is shown in Fig. 1a. Timolol produces a smooth displacement curve which on Scatchard analysis (Fig. 1b) is best described by a single straight line, the slope of which represents the IC_{50} value. In contrast, in a similar analysis for practolol (a selective β_1 antagonist)¹² the displacement curve clearly shows an inflection at lower concentrations of the antagonist (Fig. 2). The Scatchard analysis of this data cannot be represented by a single straight line. This suggests either the presence of more than one binding site or negative cooperativity. The latter phenomenon is unlikely, as the inflection in the Scatchard plot is only observed with the β_1 selective agents in these membranes. In addition, the Hill plot of the same data (not shown) does not yield a single line of low slope, but gives two separate components. Consequently, the Scatchard plot (Fig 2b) is best satisfied by two lines suggesting the presence of two binding sites with different affinities for practolol. One set of sites has a low affinity (line A) and the other a high affinity (line B) for this β_1 antagonist. Similar experiments have been carried out using propranolol, oxprenolol, atenolol and para-oxprenolol. Again only the β_1 -selective antagonists atenolol¹³ and para-oxprenolol⁷ show the presence of the two binding sites, with line B in each case intercepting at about 25% on the y axis of the Scatchard plot.

We have used membranes prepared from rat heart as a potential source of relatively pure β_1 receptors. Displacement of ^3H -DHA from heart membranes by β_1 antagonists suggests that the majority (> 80%) of the receptors have a high affinity for these compounds. As

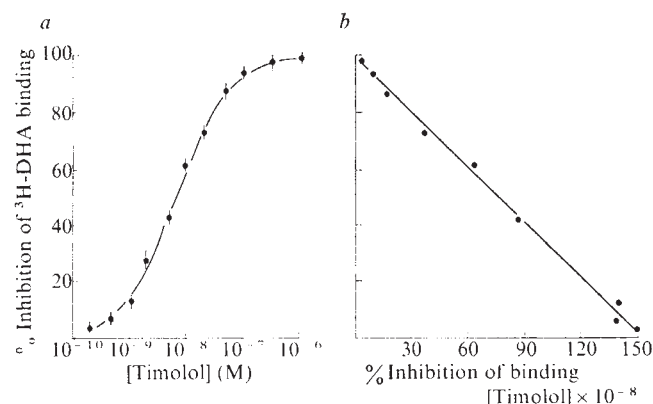


Fig. 1 a, Inhibition of ^3H -DHA binding to rat lung membranes by (—)timolol. Lungs were removed from male Wistar rats (100–150 g) and dissected free of major bronchi. Tissues were homogenised in a buffer containing Tris-HCl 5 mM (pH 7.4), sucrose 0.25 M and MgCl_2 1 mM, using a glass Teflon homogeniser. Crude membranes were separated by centrifugation at 480g for 10 min. The supernatant was then re-centrifuged at 30,000g for 10 min. The final pellet was washed three times before resuspension in assay buffer at a concentration of 100–200 μg protein per 100 μl . Membranes were incubated in a final assay volume of 250 μl which contained Tris-HCl, 50 mM (pH 7.8), 8,000–12,000 c.p.m. (1 nM), (—) ^3H -DHA and various concentrations of the antagonist. All incubations were performed for 30 min at room temperature and were terminated by vacuum filtration through Whatman GFB glass fibre filters and washing with 3×5 ml of incubation buffer. Filters were counted in a Triton-toluene-based scintillation fluid and counted in a Packard liquid scintillation spectrometer at an efficiency of 40%. Separate incubations were also carried out in the presence of 200 μM isoprenaline to determine nonspecific binding and in all cases specific binding was taken as total minus nonspecific binding. Results shown are the means of duplicate estimations in three separate experiments ($\pm$ s.e.m.). **b**, Scatchard analysis of the data presented in (a). Slope of the line shown is the IC_{50} value for (—) timolol, 6×10^{-9} M.

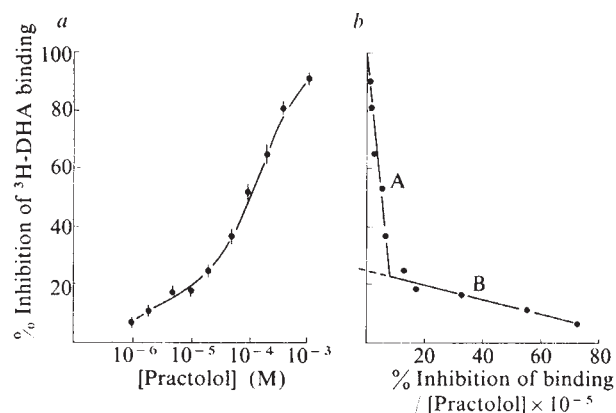


Fig. 2 a, Inhibition of ^3H -DHA binding to rat lung membranes by ($\pm$) practolol. Incubations were carried out as for (—)timolol (Fig. 1a). Results shown are the means of duplicate estimations in three separate experiments ($\pm$ s.e.m.). **b**, Scatchard analysis of the data presented in (2a). Slopes of lines A and B depicted are the IC_{50} 's of each component. For line A, $IC_{50} = 2.4 \times 10^{-4}$ M; for B, 2.6×10^{-6} M.

there is a relatively low number of binding sites in cardiac tissue (10–20% of those in lung), the possibility of a small number of low affinity sites in this tissue cannot be eliminated. However, it is possible to use the heart as a reference tissue for the binding affinities of the β_1 antagonists. Table 1 summarises the K_i values (inhibition constants) for the antagonists tested in displacement experiments using lung and heart membranes. As expected lung and heart exhibit the same K_i values for nonselective compounds and similar stereoselectivity. With the selective β_1 antagonists however, the K_i values calculated from the slope of line B in the lung were very similar to the K_i values for these compounds in the heart, whereas the K_i from line A shows the expected difference from heart related to the β_1 selectivity of the compound.

It has been suggested that β adrenoreceptors might, in analogy to isoenzymes, be considered as isoreceptors having varying affinities for β adrenoreceptor agonists and antagonists^{14,15}. This hypothesis has arisen from findings that agonists and antagonists seem to have a spectrum of affinities depending on the tissue and species examined^{3,4}. There are contradictory pharmacological data which suggest the existence of only two receptor subtypes present in varying proportions in different tissues^{16,17}. This might

Table 1 K_i values for β -adrenergic antagonists determined by inhibition of ^3H -dihydroalprenolol binding to rat lung and heart membranes

	K_i (M)	
	Lung	Heart*
(—)Propranolol	5×10^{-10}	3×10^{-10}
(+)Propranolol	3×10^{-8}	3.9×10^{-8}
(—)Timolol	1.5×10^{-9}	1.25×10^{-9}
($\pm$)Oxprenolol	1×10^{-9}	1.3×10^{-9}
	A	
($\pm$)Practolol	6×10^{-6}	6.5×10^{-7}
($\pm$)Atenolol	2.9×10^{-6}	5×10^{-7}
($\pm$)Para-oxprenolol	4×10^{-6}	5×10^{-8}
	B	
($\pm$)Practolol	6×10^{-6}	8.6×10^{-7}
($\pm$)Atenolol	2.9×10^{-6}	2×10^{-7}
($\pm$)Para-oxprenolol	4×10^{-6}	1×10^{-7}

K_i is determined from the equation $K_i = IC_{50}/(1 + S/K_D)$ where S is the concentration of ^3H -DHA used in the assay (0.5–1.5 nM) and $K_D = 0.5$ nM. The results are the means of at least three separate experiments (s.e.m. in each case < $\pm 5\%$).

* Heart membranes prepared and assayed as for lung (Fig. 1a) had a K_D for ^3H -DHA that was not significantly different from that in the lung.

explain the apparent spectrum of affinities for various agents found by the groups supporting the 'isoreceptor' hypothesis. However, all these results have been obtained from intact preparations and it is appreciated that the analysis of ligand-receptor interactions in whole tissue is prone to a number of serious limitations^{18,19}. Thus, for example, there may be differing rates of loss of agonist by nonspecific binding and uptake, or the time for equilibration of drugs between bulk and receptor phase may vary between preparations. The use of direct receptor binding studies in purified membrane fragments should circumvent most of these problems and the present studies are the first to suggest that even at this level only two distinct β adrenoreceptor binding sites can be demonstrated in the same tissue.

The presence of a heterogeneous population of β adrenoreceptors is not limited to lung membranes, as similar results have been obtained using direct binding techniques in rat brain (submitted for publication). Thus in rat cerebral cortex β_1 -selective agents such as atenolol and practolol have high affinity for about 65% of the receptors but considerably lower affinity for the remaining binding sites. For example, the K_i values of practolol for the two sites (A and B) in rat cerebral cortex are 6.5×10^{-5} M and 2.86×10^{-7} M respectively. These values closely agree with those in rat lung (Table 1) although clearly the proportions of high and low affinity sites differ considerably between the two tissues. This emphasises our belief that there are only two β -adrenoreceptor subtypes as was originally suggested by Lands *et al.*¹ but that they may exist in varying proportions in different tissues.

The presence of both β_1 and β_2 binding sites in these tissues may be related to heterogeneous cell populations and it will be important to determine whether both receptor subtypes serve similar physiological functions. Studies on the cellular localisation of β receptor binding sites and on the possible alteration in the ratio of the receptor subtypes in response to hormonal or pharmacological intervention are under way.

D. B. BARNETT

ELIZABETH L. RUGG

S. R. NAHORSKI*

Department of Pharmacology and Therapeutics,
Medical Sciences Building, University of Leicester,
University Road, Leicester, UK

Received 31 October 1977; accepted 9 March 1978.

* To whom correspondence should be addressed.

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Analogues in other mammals and in fish of human plasma proteins, C-reactive protein and amyloid P component

Two apparently disparate human plasma proteins, C-reactive protein (CRP) and amyloid P component (protein SAP)¹, have been shown to have substantial homology of amino acid sequence, similar subunit composition and similar molecular appearance in the electron microscope². We now report for the first time the isolation (using calcium-dependent affinity chromatography) of proteins closely resembling human SAP, from sera of other mammals, amphibia and fish, and of proteins from the plaice (*Pleuronectes platessa* L.) and the chicken, which closely resemble human CRP. The stable evolutionary conservation in this plasma protein family of calcium-dependent ligand-binding specificity and molecular architecture during much of vertebrate evolution implies that they have important functions, and also facilitates their experimental investigation.

CRP is the prototype acute phase reactant, its plasma level increasing by as much as 1,000-fold from a normal value of less than $2 \mu\text{g ml}^{-1}$ within hours of onset of most tissue damaging inflammatory processes³⁻⁵. CRP undergoes calcium-dependent binding to phosphoryl choline residues of phospholipids such as lecithin and sphingomyelin⁶, and once complexed it activates the classical complement pathway⁷⁻¹⁰. CRP has also been reported to enhance phagocytosis¹¹, inhibit platelet function^{12,13} and antigen-induced T-cell activation¹⁴⁻¹⁶, but despite these *in vitro* properties its *in vivo* role is still uncertain.

In contrast to CRP the human serum concentration of protein SAP is relatively constant (mean $\pm$ s.d. $37 \pm 13 \mu\text{g ml}^{-1}$ in 76 healthy adults) and varies little with disease¹⁷. A possible physiological ligand for SAP *in vivo* has not been identified, nor has its function; but its presence in amyloid deposits (from which it was first isolated) may be explained by its calcium-dependent binding to amyloid fibrils (M.B.P., M. Skinner and A. S. Cohen, unpublished). The claim that protein SAP was a fourth subcomponent (Clt) of Cl (refs 18 and 19) was incorrect and has now been withdrawn²⁰.

Analogues of human SAP were obtained from the sera of the species listed in Table 1 using the method we have recently described for preparation of human SAP²¹. The technique depends on the calcium-dependent adherence of protein SAP to Sepharose 4B beads (Pharmacia). After incubation of serum with the beads and thorough washing with calcium-containing Tris-buffered saline, protein SAP was eluted with EDTA or citrate. In some cases it was further purified by gel filtration on Ultrogel AcA34 or AcA44 (LKB Instruments, Ltd.). Human and rabbit CRP were isolated from pathological plasma, serum or exudate fluids as described previously using calcium-dependent affinity chromatography on Sepharose-C-polysaccharide beads, and freed from contaminating SAP by gel filtration on Ultrogel AcA44 in the presence of calcium ions²². CRP-like proteins from pooled normal plaice sera²³ and from acute phase chicken sera were obtained by the same method.

When purified human SAP and CRP were examined in the presence of EDTA in gradient polyacrylamide gel electrophoresis (PAGE) (PAA 4/30 gels Pharmacia)²⁴ and compared with markers of known molecular weight (apoferritin, catalase and caeruloplasmin) their apparent molecular weights were $290,000 \pm 5\%$ and $161,000 \pm 7\%$ respectively. The molecular weight of protein SAP measured using sedimentation equilibrium analysis²⁵ in the presence of EDTA was 255,000, a value in reasonable agreement with the 233,000 recently reported by Painter¹⁹. CRP was hetero-

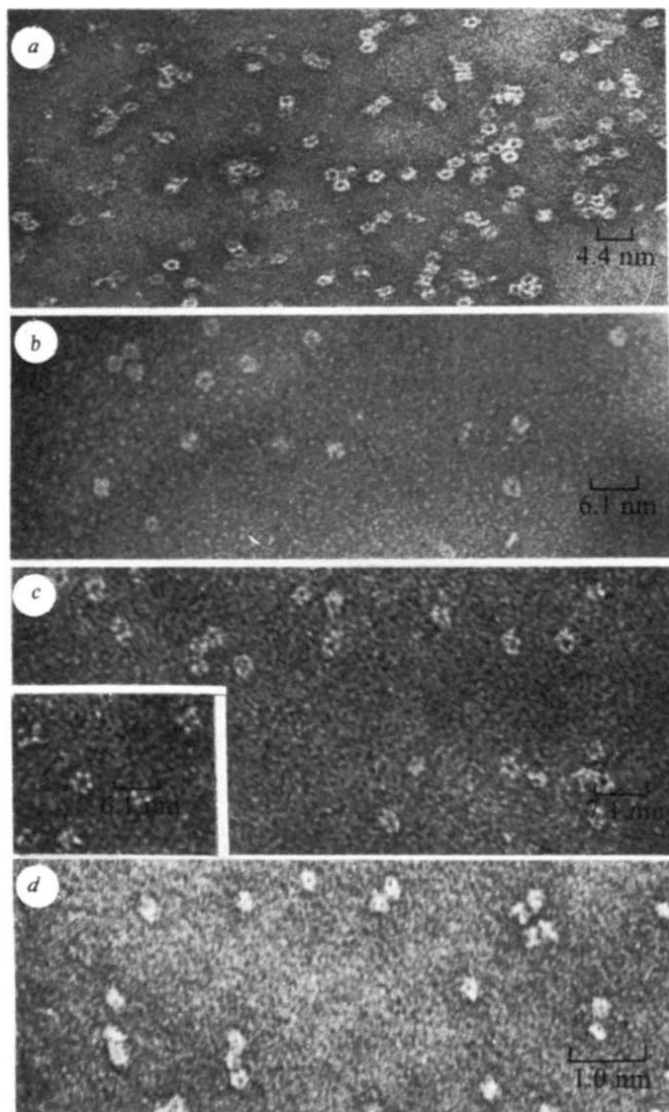


Fig. 1 Electron micrographs of protein SAP molecules negatively stained with 2% sodium silicotungstate (pH 7.3). a, Human; b, bovine; c, plaice (inset: flounder); d, dogfish. Scale bar represents approximately 20 nm.

geneous in the ultracentrifuge as described previously²⁶, with one component of molecular weight 107,000 and another of 130,000. The anomalous behaviour of these proteins in gradient polyacrylamide gel electrophoresis (PAGE) must be due to their known molecular asymmetry. Of the proteins isolated by virtue of their calcium-dependent binding to Sepharose those from plaice, flounder and rabbit ran almost identically with human protein SAP in gradient PAGE; pig, mouse and dogfish ran in a lower molecular weight position similar to human CRP, whereas cow and goat both had an apparent molecular weight of $213,000 \pm 8\%$. The molecular weights of the plaice and dogfish proteins determined using sedimentation equilibrium analysis were 246,000 and 135,000, confirming the similarity in size to human SAP and CRP, respectively.

Electron micrographs of human protein SAP (Fig. 1a) confirm the appearance described previously^{19,27} and interpreted as two pentameric discs interacting face to face¹⁹. Proteins isolated on Sepharose from other mammalian sera (rabbit, ox and pig) seemed to be similar to human SAP in the electron microscope (see, for example, Fig. 1a and b). The corresponding proteins from the two teleosts (plaice and flounder) also closely resembled the mammalian molecules (Fig. 1c). However, the protein from the dogfish

seemed to be more compact (Fig. 1d) with a smaller central hole.

All the Sepharose-binding proteins formed single bands after SDS-PAGE indicating that each was composed of subunits of a single size. The apparent molecular weights in reducing gels fell within the range 19,000–27,000 (Table 1). The subunits interact non-covalently since dissociation was found in gels without reducing agent. The apparent subunit size was similar with and without reduction, suggesting that there is no extensive intra-subunit disulphide bridging.

In summary, evidence from our PAGE, ultracentrifuge and electron microscope studies, suggests that the proteins in all these diverse species are analogous to one another and to human SAP, with the same ligand specificity and similar subunit size. Although in plaice, flounder and rabbit the size of the whole molecule and the arrangement of subunits was, in the conditions of examination, very similar to human SAP, it was clear that this could not be the case with dogfish, pig and mouse which more closely resembled human CRP in size.

Proteins isolated in trace amount ($1\text{--}2\text{ }\mu\text{g ml}^{-1}$) from pooled normal dogfish and flounder sera by their calcium-dependent binding to pneumococcal C-polysaccharide (CPS) had apparent molecular weights very similar to human CRP

Table 1 Molecular weights of subunits in SDS-PAGE

Species	Protein SAP	CRP
Marine toad, <i>Bufo marinus</i>	27,300	
Plaice, <i>Pleuronectes platessa</i> L.	25,600	18,600
Mouse	25,300	
Pig	24,400	
Cow	24,000	
Human	23,650	21,100
Donkey	21,600	
Goat	21,100	
Sheep	21,100	
Flounder, <i>Platichthys flesus</i> L.	21,100	
Rabbit	19,950	18,900
Dogfish, <i>Scyliorhinus caniculus</i>	19,300	
Chicken	Not done	21,100

Three different gel systems were used^{29–31}. With the same calibrating proteins (carboxypeptidase, carbonic anhydrase, chymotrypsinogen, kappa light chain, trypsin, β -lactoglobulin, myoglobin and lysozyme) it was found that the molecular weights of protein SAP and CRP subunits depended on the gel system used. However, the relative molecular weights were the same in different systems and in the table have been normalised to the known molecular weight of human CRP²⁸. In view of the lack of agreement between our own (M.B.P. and J. E. Fox, unpublished) and the previously reported³² content of carbohydrate in human protein SAP, and the absence of any information on carbohydrate content of the animal analogues, it is impossible to assess its contribution to the apparent molecular weights estimated using SDS-PAGE.

in gradient PAGE. Insufficient amounts of CPS-binding proteins were available from flounder and dogfish for electron microscope or SDS-PAGE examination. In contrast substantial quantities of CPS-binding protein were present in pooled normal plaice serum (mean $\pm$ s.d., $55 \pm 8\text{ }\mu\text{g ml}^{-1}$). Two batches isolated from different pools of plaice serum gave five distinct bands in gradient PAGE with apparent molecular weights of $256,000 \pm 1\%$, $247,000 \pm 2\%$, $226,000 \pm 2\%$, $205,000 \pm 2\%$ and $189,000 \pm 1\%$. Despite this heterogeneity, the plaice CRP molecules closely resembled those of human and rabbit CRP in the electron microscope (Fig. 2a–c). They did differ, however, in their pronounced tendency to form stacks (Fig. 2c), a characteristic of protein SAP¹⁹. The subunits of human, rabbit and plaice CRP also behaved similarly in SDS-PAGE with apparent molecular weights of 18,000–21,000 (Table 1). These were unaffected by reduction, as expected in the case of human CRP which is now known to have only one intrachain disulphide bridge

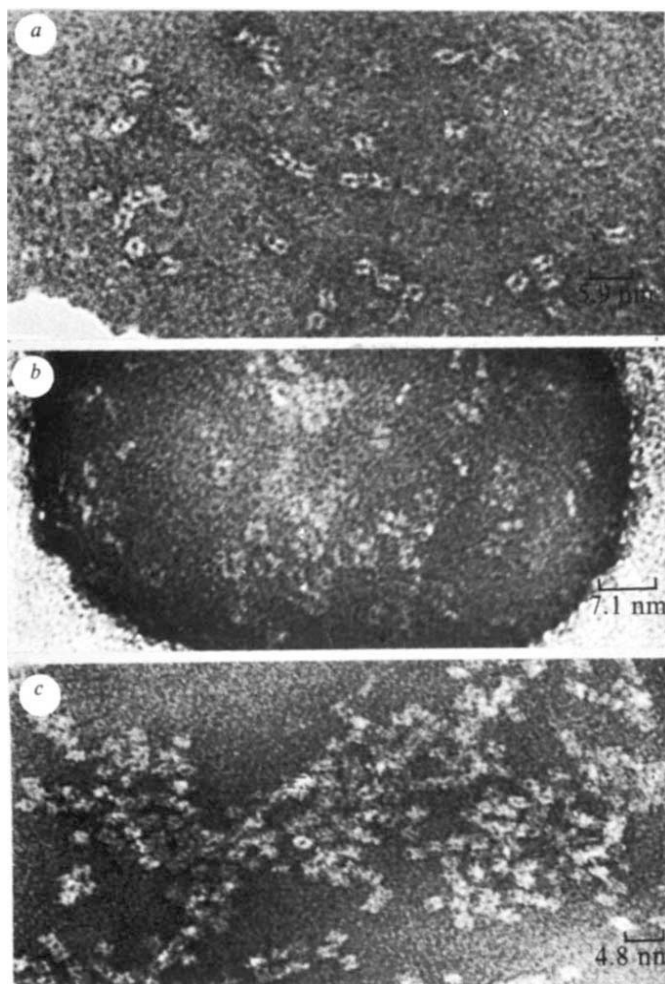


Fig. 2 Electron micrographs of CRP molecules negatively stained with 2% sodium silicotungstate (pH 7.3). a, Human; b, rabbit; c, plaice. Scale bar represents approximately 20 nm.

between residues 36 and 78 (ref. 28). A calcium-dependent CPS-binding protein was isolated from acute phase chicken sera (obtained 24 h after subcutaneous injection of croton oil), but not from normal chicken sera. The apparent sizes of the whole molecule in gradient PAGE and of the subunits in SDS-PAGE were the same as human CRP; their appearances in the electron microscope were also very similar.

Our results indicate that the plaice and chicken CPS-binding proteins are analogues of mammalian CRP, with similar subunit structure, molecular arrangement and ligand specificity. The source of the heterogeneity of plaice CRP seen in gradient PAGE is not clear, and neither is the reason for its presence in appreciable amounts in all the pools of serum from apparently healthy plaice examined so far. This differs strikingly from mammalian and chicken CRP and from the situation in the closely related flounder, resembling more the stable normal circulating level of protein SAP (ref. 17). These phenomena are under investigation.

In conclusion we have demonstrated that proteins analogous to human CRP and SAP are present in a variety of lower animals. Confirmation of homology by amino acid sequence is in progress. The widespread occurrence and stable conservation of these proteins suggest that their *in vivo* functions have warranted their persistence in evolution without marked modification of structure, and provide the opportunity for further experimental investigation of these functions.

This work was supported in part by the MRC. We thank Dr J. Ivanyi for providing the acute phase chicken sera, and Mr M. Edwards for technical assistance.

M. B. PEPYS

A. C. DASH

Immunological Medicine Unit,
Department of Medicine,
Royal Postgraduate Medical School,
London W12 0HS, UK

THELMA C. FLETCHER

NERC,
Institute of Marine Biochemistry,
Aberdeen, UK

N. RICHARDSON

E. A. MUNN

A. FEINSTEIN

Department of Immunology,
ARC Institute of Animal Physiology,
Babraham, Cambridge, UK

Received 4 November 1977; accepted 10 March 1978.

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Errata

In the letter 'Faint X-ray sources detected near COS B γ -ray positions' by P. F. Julien and H. F. Helmken, *Nature* **272**, 699, in line 4 paragraph 1 for CB185-5 read CG185-5. In Table 1 footnote for CG195 $\pm$ 4 read CG195+4. In Fig. 1b the x axis should read 1 Nov 71 to 1 Feb 73.

In the review article 'The cosmic ray isotopes' by P. Meyer *Nature* **272**, 675, on page 678, line 11 should read . . . 0.2 hydrogen atoms cm⁻³ . . . (not cm³). Line 44 in the right-hand column should read . . . per nucleon particles.

In the letter 'Irreversibilities in the mechanism of photo-electrolysis' *Nature* **271**, 137 the name of the first author was incorrectly spelt. It should read Ferd Williams.

In the letter 'Single crystal analysis of the structure of stishovite' by W. Sinclair and A. E. Ringwood in paragraph 4 line 2 the space group should read P4₂/m 2₁/n 2/m.

reviews

DNA insertions

William Hayes

DNA: Insertion Elements, Plasmids and Episomes. Edited by A. I. Bukhari, J. A. Shapiro and S. L. Adhya. Pp. 782. (Cold Spring Harbor Laboratory: New York, 1977.) \$36.

THIS book deals in detail with one of the most interesting and important discoveries to emerge from molecular genetics during the past twenty-five years, and signifies a landmark in the history of genetics and of evolution in general. About ten years ago a new class of reversible mutations was found. These mutations, involving a number of *Escherichia coli* operons, were highly polar, suppressing the expression of promoter-distal genes of operons, but were not due to deletions, frame-shifts or base substitutions. Similar mutations were found to occur in F-prime plasmids and also in certain transducing phages in which the mutations led to an increase in buoyant density, whereas reversion restored the density to normal. It was concluded that the mutations were due to insertions of extraneous DNA fragments into the bacterial DNA. The fact that the mutations caused by these 'insertion sequences' (ISs) could revert to normal function implied that they were capable of precise excision. Subsequently it has been shown that mutations of this sort comprise a considerable proportion of all spontaneous mutations in bacteria, phages and plasmids.

It often happens that discoveries of new phenomena coincide with the independent development of new techniques for their elucidation. In particular, the use of electron microscope heteroduplex analysis of DNA not only confirmed the existence of insertion sequences but, in conjunction with genetic analysis, soon revealed features of structure and behaviour which suggested that the role of ISs in nature might go far beyond that of mere mutator elements.

These elements comprise many diverse DNA sequences ranging from 800 to many thousand base-pairs long, but in bacteria four distinct elements are common. They may be located at many different sites in the same bacterial chromosome, may be inserted in either orientation and can transpose from one site to another in the

absence of the normal bacterial recombination system (that is, in *recA* mutants). If inserted into an operon, two of these elements (IS1 and IS4) yield polar mutations irrespective of orientation, whereas the other two (IS2 and IS3) are mutagenic in one orientation only. In its non-polar orientation IS2 acts as a strong promoter and, if inserted into an operon, can transform an inducible system into a constitutive one. Yet another element carries a recognition region for the transcription-terminating protein ρ . Moreover some IS elements, while remaining *in situ*, can induce repeated deletions of DNA adjacent to one of their extremities so that genes located at the other extremity may be brought under the control of new bacterial promoters. Thus these elements, which have been found in many bacterial genera, must have exerted a profound evolutionary influence far beyond that of mutation alone.

However, the most far-reaching implications of ISs stem from their role in promoting interactions between the genomes of bacteria, plasmids and phages, and in the formation of R factors—those ubiquitous transmissible plasmids responsible for the world-wide dissemination among bacteria of multiple drug-resistance determinants. It turns out that the property of the fertility plasmid F to insert into many possible sites on the *E. coli* chromosome, to form different Hfr cells, is in fact due to a *recA*-independent reciprocal recombination event between the same IS element present in the two genomes. Heteroduplex analysis reveals that the inserted plasmid is bounded by a pair of these sequences. Similarly in F-prime plasmids, which are derived from Hfr cells and have incorporated an adjacent region of chromosome, the junctions between bacterial and plasmid DNA are marked by a pair of ISs of the same type, which have thus mediated the transposition. By implication, interaction between ISs at different chromosomal locations may, in the absence of plasmids, cause deletions (which are often region-specific and start at a precise point), transpositions, and inversions if sequences in opposite orientation interact.

Transmissible drug resistance has, because of its medical importance, for many years been the topic of much research. Recent work has shown that the determinants of resistance to at least seven different antibiotics form part of independent structures several kilobase-pairs long, called 'transposons', which can jump between the genomes of bacteria, plasmids and phages of widely different base compositions and insert themselves at many sites in the absence of *recA* function, sometimes producing mutations or inducing deletions. As in the case of F-prime factors, the ends of any given inserted transposon are bounded by identical sequences, as judged by heteroduplex analysis. In some cases these are homologous to known IS elements. Sometimes these sequences are similarly orientated but more often they are inverted (palindromic) so that, like some ISs, heteroduplexes, with homologous DNA segments lacking the transposon, show a characteristic 'lollipop' structure in which a long single-stranded loop terminates in a short double-stranded stem. As neither ISs nor transposons have yet been found to exist in circular form, it is assumed that the two ends of the linear DNA molecules are inserted between adjacent base pairs by an enzyme encoded in the molecules themselves and by a mechanism as yet not understood.

A more complicated variant of these elements, displaying all the main features of the ISs, is the genome of the mutator phage Mu, discovered 15 years ago, which can insert itself indiscriminately into the DNA of a wide range of bacterial genera, plasmids and phages. This phage offers not only a simple system for the analysis of IS phenomena, but also a tool for *in vivo* genetic engineering because of its ability to transpose chromosomal regions from one bacterial genus to another.

One of the most exciting prospects of these new discoveries is that they may well explain hitherto mysterious phenomena in eukaryotes. Such phenomena embrace the transposable controlling elements in maize first described by Barbara McClintock 25 years ago, the anomalous behaviour of certain mutations in *Drosophila* that

mimic insertion mutations, mating type interconversions in yeast, transpositions and inversions in general, and the insertion into host genomes of some DNA viruses and DNA copies of RNA viruses. Although most of the evidence for involvement of ISs in such occurrences is circumstantial, many inverted repeat (palindromic) sequences have been found in the DNA of all eukaryotes examined and, as in transposons, seem to flank unique sequences in the genomes of certain viruses.

The book reviewed here, containing 66 original papers, was inspired by a broadly based meeting on DNA insertions held at the Cold Spring Harbor Laboratory in May 1976 but includes many articles more recent than those then presented. These articles are organised into six main sections: "IS Elements"; "Transposons and Plasmids"; "Bacteriophage Mu"; "Non-Homologous Recombination and the λ Paradigm"; "Eukaryotic Systems", and a more practical section on "Genetic Rearrangements: Techniques and Applications". In addition, the final 185 pages of the book are devoted to four Appendices providing complete and intricate details of genetic, physical and restriction enzyme maps and other technical information concerning all the relevant and important IS elements, plasmids and phages, as well as DNA sequence analyses of IS elements and recognition

sequences for restriction enzymes. All these appendices, and most of the papers, have an extensive list of references.

Although the book is an essential and unique one for those working in this exciting new field of molecular biology, it has many features which improve its appeal to the more general geneticist. There is, for example, an excellent and clearly written introduction by the editors, followed by a chapter on nomenclature which renders some of the subsequent jargon more comprehensible. Each Section is preceded by an illustration typifying its contents, with a simple explanation of its meaning on the reverse, and nearly all the papers end with a lucid and informative "Concluding Remarks" and "Summary", so that the essence of their contents, sometimes repetitive and usually highly technical, may readily be understood by the uninited.

The editors are to be congratulated on their initiative in producing such a book, on their selection and organisation of its material and on the uniformity of presentation that they have clearly imposed on its many contributors. At \$36 the book is moderately priced. □

William Hayes is Professor of Genetics at the Australian National University, Canberra.

Arthropod morphology and evolution

The Arthropoda: Habits, Functional Morphology and Evolution. By S. M. Manton. Pp. 527. (Oxford University Press: Oxford, London and New York, 1977.) £20.

FOR half a century Dr Sidnie Manton has pursued high quality studies on the functional morphology of a wide variety of arthropods, the evolutionary implications of which have been of profound significance to students of this vast and diverse assemblage. Now, undeterred by arthritis and cataracts, she has produced a book which synthesises some of her many complex studies, supplements them with recently acquired palaeontological information, and proclaims what she regards as the major phyletic deductions to be drawn from them. In so doing she has rendered a valuable service.

The book presents a splendid panorama of arthropod morphology, interpreted in the light of function, and of the abilities and achievements that depend on these

morphological attributes. Such wide-ranging treatment as is presented would have been impossible but for her own studies. As few can have read in detail the long and complex papers in which many of the facts reported first saw the light of day, some of the achievements of these remarkable animals will come as a revelation to many. Whereas the Onychophora show what can be done with a deformable scute-free body and lobopodial legs, and whereas other groups, such as the Cladocera among the Crustacea, have exploited other features, the major successes of the arthropods have been based on an armoured, segmented trunk, jointed legs, and cephalic elaboration, the exploitation of which has led to almost endless diversity.

In learning about these successes we follow the evolution of crustacean mandibles from the primitive, yet complex, rolling appendages of branchiopods to the transversely biting jaws of the 'higher' Malacostraca; learn about such remarkable features of the Myriapoda as the sliding sternites by means of which certain diplopods are enabled to enhance the range and strength of leg movements; of the anterior tentorial apodemes with which myriapods abduct their mandibles;

of the significance of tergite heteronomy in fast-running chilopods; of the way gaits change with speed of running in such animals, only a few of whose legs are involved in propulsion at any given time during fast running; of contrasting attributes in slower-moving diplopods, such as the way in which maximum thrust is achieved by many simultaneously pushing legs during burrowing; of synovial cavities (until recently known only in vertebrates) in the joints of certain diplopods; of the significance of diplosegments; and more, much more, besides. Flight, so important in the Pterygota, is not treated in detail but the origin of flight mechanisms and the evolutionary history of flight muscles are discussed.

Dead animals would usually have provided the morphological facts, but the significance of these could often be revealed only by observing habits and abilities in life, and it is by such observations that our real understanding is being vastly advanced. (Counters and measurers of carcasses take note.) The morphology itself is dealt with in admirable detail, but never in a manner detached from function, save in the case of fossils from which intelligent deductions can now sometimes be made, with due caution, in the light of our knowledge of living animals. The whole morphology of onychophorans, that enables them to live as they do, the nature of the jaws in various groups, salient morphological features of xiphosurans, muscular systems of myriapods, leg structure in a wide range of uniramiens and the nature of the coxa-body joints to be found therein, and many other matters, are dealt with by presenting a mass of concrete facts, mostly in a palatable form.

To be of value, presentation of such information is heavily dependent on adequate and accurate illustrations, of which there are many, and it is tragic—this is not too strong a word—that in a large number of cases references to these figures or vital points of labelling should have been confused. Readers directed to the wrong figure or the wrong part of a figure will be bewildered; and one wonders what devil was at work that caused the labelling of adductor and abductor muscles to be transposed in certain figures. With the labour of turning literally thousands of times to illustrations (I counted 36 references to figures on one page), this calamity, for which the publisher must surely be at least partially responsible, will deter and confuse readers of this unique contribution to our understanding of arthropods.

The flood of recently acquired information inevitably has phyletic implications. Recognition of the onychophorans, myriapods and hexapods as a group which the author believes should be regarded as a distinct phylum, the Uniramia, is one of these. Another important conclusion is that the Arthropoda is not, as once

supposed, a monophyletic group derived from annelids but a polyphyletic assemblage the ancestry of which is obscure. The immense problem of locating ancestral forms is evident when one considers the diversity of arthropods, some displaying considerable complexity, and often of problematic affinities, already differentiated by Cambrian times. The concept of a polyphyletic origin, already supported by embryological studies, implies a great deal of convergence, but will surely win the day.

Manton goes further than this and believes that the hexapods do not represent a monophyletic assemblage but probably have a fivefold origin, the Collembola, Protura, Diplura, Thysanura and Pterygota being independently evolved from multilegged ancestors with a head fundamentally different from that of myriapods. Such a view will meet with resistance from some entomologists; and already, for example, Kristensen (*Z. Zool. Syst. Evolut.-Forsch.* 13, 1-44; 1975) has disagreed with such a conclusion. He also persists in using the term Entognatha as a taxonomic unit, which surely weakens his case. Manton believes that entognathy should be used as a purely descriptive term of no phyletic significance. That it could have arisen independently in different groups of hexapods is suggested by the fact that it has done so in copepods

and isopods, both groups of which crustaceans display varying degrees of its acquisition according to functional demands.

Kristensen views hexapod evolution from within what, whatever its merits, some would regard as the straight-jacket of Hennigian phylogenetic systematics, whereas Manton takes a broader view. It would have been pleasing, however, if some of his well reasoned arguments concerning the possible existence of stages that Manton believes to have been functionally impossible had been specifically considered. Presumably these were not available in time. One looks forward to a continuation of this debate.

Of any such work there are bound to be minor criticisms. For example, even Manton momentarily forgets that branchiopod crustaceans do not have calcified cuticles, and that certain attributes of calanoid copepods are not shared by cyclopoids. Such minor points are, however, overshadowed by the labelling problems already mentioned which mar what, irrespective of one's views on its phylogenetic conclusions, must be hailed as one of the most significant works on arthropods ever published.

Geoffrey Fryer

Geoffrey Fryer is a Senior Principal Scientific Officer at the Windermere Laboratory of the Freshwater Biological Association, Ambleside, Cumbria, UK.

Soil classification handbook

Quantitative and Numerical Methods in Soil Classification and Survey. By R. Webster. Pp. 269. (Oxford University Press: Oxford, New York and London, 1977.) £12.50.

ALTHOUGH the use of numerical and quantitative methods of data analysis have been a feature of the biological sciences for many years, until recently they have been little used in the mapping or classification of soils. There have been several reasons for this, not the least being the absence of a suitable handbook giving guidance not only with the design of information gathering procedures which would yield data amenable to numerical analysis, but also with the techniques of analysis most suited to such data. With the publication of this excellent book Dr Webster, one of the leading experts on numerical methods in soil science, has provided in one volume just such guidance.

Within the book the author has commendably achieved a balance between the need for statistical theory and the need for the text to be intelligible to the non-

mathematical soil scientist. The text takes the reader through a number of introductory chapters covering data handling, problems of measurement and description, sampling and estimation, generalisation and nested classification. From there the author moves on to consider the more sophisticated techniques of multivariate analysis involving ordination and classification, with a final chapter on mapping techniques. Each procedure is discussed in detail, with valuable comments on its applications and limitations. Discussion is further enhanced by the inclusion of worked examples of field data. These examples are invaluable in aiding the comprehension of some of the more complex multivariate methods where the text at times is too concise. Even in some of the examples (at least to the uninitiated) figures are apparently derived from nowhere; one such example is the calculation of latent vectors; here the inclusion of the solution to a quadratic would have been of immense value.

These minor criticisms apart, this book should be essential reading for all those seeking guidance in the application of quantitative methods in soil classification and survey.

M. J. Alexander

M. J. Alexander is Lecturer in Geography at the University of Durham, UK.

Encyclopaedia of the platyrrhines

Living New World Monkeys (Platyrrhini), with an Introduction to Primates. Vol. 1. By P. Hershkovitz. Pp. 1117. (University of Chicago Press: Chicago and London, 1977.) \$80; £55.

THE separation between the New World monkeys (platyrrhines) and the Old World monkeys and apes (catarrhines) is an ancient one. The two groups diverged from pre-simian stock sometime between the end of the Cretaceous and the Eocene, and their living representatives show a wide variety of morphological and physiological differences. Partly because they are less closely related to man and partly because of the limited data available on South American species, the platyrrhines have attracted less attention than the catarrhines, and their taxonomy has long been in serious disarray. This is particularly true of the Callitrichidae (marmosets and tamarins), the smaller and more primitive of the two main existing families of platyrrhines. Hershkovitz's enormous monograph provides an authoritative summary of available knowledge of the morphology, biology and phylogeny of the fourteen existing callitrichid species as well as of *Callimico* (Goeldi's monkey), which he assigns to the monospecific family, Callimiconidae. The review of the literature is encyclopaedic and particular attention is paid to areas (including comparative physiology, behaviour and ecology) often ignored by comparative anatomists. For many years, this will certainly remain the most important reference work on callitrichids.

Modern evolutionary biologists are likely to be disturbed by Hershkovitz's emphasis on irreversible evolutionary trends in colouration. The coats of 'primitive' species are usually agouti-coloured—an effect produced by alternating bands of blackish brown and reddish colour on the terminal half of each hair. Subsequent evolution, Hershkovitz argues, progresses through all-reddish or all-blackish forms and follows "an orderly and irreversible sequence of degeneration that ends in loss of pigment or white" (p94). Mostly white or black-and-white colouring is typical of "advanced" species and of those occurring furthest from the origin of the group. The theory is difficult to accept—both because there is no firm functional reason for predicting that evolution should take this course and because the evidence (apart from colouration) on which species or races are classified as "primitive" or "advanced" is difficult to interpret in the virtual absence of a fossil record. Nevertheless, it is clear that clinal colour variation is not closely related to obvious environmental parameters: not only do

different races occupying precisely similar habitats show strikingly different colouration, but colour clines run in different directions in different groups. The topic is a fascinating one, though it has been extensively neglected. The erection of Herskovitz's hypothesis that colour change is constant in direction and irreversible should stimulate evolutionary

biologists to examine the ecological correlates of colouration in an attempt to find a more satisfactory explanation.

T. H. Clutton-Brock

T. H. Clutton-Brock is a Senior Research Fellow in the Behavioural Ecology Group of the King's College Research Centre, University of Cambridge, UK.

Seed dormancy and germination

The Physiology and Biochemistry of Seed Dormancy and Germination. Edited by A. A. Khan. Pp. 447. (Elsevier/North-Holland: Amsterdam, New York and Oxford, 1977.) \$65.95; Dfl.162.

THIS multiauthor volume must surely become regarded as the standard text on this subject, at least for the time being. The editor has managed to bring together a more representative and distinguished panel of authors than had previously been achieved in this area, and most of the contributions are of a very high standard, written in a clear style with commendable economy of words. Particularly outstanding are a gem of an prefatory chapter by K. V. Thimann and a review of the effects of gibberellin on barley aleurone tissue, in the chapter by R. L. Jones and J. L. Stoddart, which summarises a weighty literature by means of two pages of text and two figures.

A weakness in the book is that the editor has allowed his contributors to define their own terms, so that the reader is able to dig out, for example, a number of different definitions of dormancy. As this may confuse anyone who reads more than one chapter, it surely would have been desirable for all of the contributors to decide on an agreed form of words for important definitions, and for these definitions to be made at the beginning of the book together with a clear statement of any differences of opinion with respect to the definitions. Confusion about dormancy arises because there are two distinct types: 'imposed dormancy' in which germination fails to occur because of the absence of suitable conditions (for example, the low moisture content of seeds in the seedsman's packet); and 'true dormancy' in which germination fails to occur in conditions which are favourable for the continued growth of the germinated seedling. In addition I deplore the use of the term 'seed' outside of its strict botanical definition; this can lead to unacceptable imprecision in scientific writing.

The first main part of the book, "General Topics", commences rather unsurely in chapters by R. C. Jann and

R. D. Amen on "What is Germination?" and by A. A. Khan on "Seed Dormancy: Changing Concepts and Theories". Their attempts to produce all-embracing hypotheses to account for dormancy and germination are less than entirely convincing, particularly as later authors emphasise the paucity of knowledge about so many aspects of this subject. M. G. Nikolaeva brings the reader back to earth with a well written chapter on "Factors Controlling the Seed Dormancy Pattern". It is a real pleasure to see that a summary account of her numerous contributions is now widely available in this form.

The second part of the book, which deals with "Hormones and Seed Germination", has been written with competence and flair, but seems to suggest that investigations on gibberellins (R. L. Jones and J. L. Stoddart) and abscisic acid (D. C. Walton) have proceeded rather more expeditiously than those on cytokinins (T. H. Thomas) and ethylene (D. L. Ketrings).

Part three commences with chapters on the effects of light and of chilling in breaking dormancy, written by W. Vidaver and by S. Lewak and R. M. Rudnicki, respectively. It seems unfortunate that the latter authors have rather overemphasised their own material, apple seed, the somewhat weak embryo dormancy of which may not be typical of seeds which require chilling. Part three closes with three good chapters on applied aspects of seed germination: one by J. D. Maguire on seed quality; one on stress by W. Heydecker, which is full of valuable information; and one by A. A. Khan on preconditioning.

Part four, on "Molecular and Metabolic Aspects of Seed Germination", opens with three excellent and concise chapters on "Nucleic Acids and Seed Germination" (D. J. Osborne), "Stored Messenger RNA and Seed Germination" (L. S. Dure, III) and "Protein Synthesis and Seed Germination" (J. D. Brooker, C. P. Cheung and A. Marcus); and continues on a high level in a chapter on "Metabolic Control of Germination" (A. M. Mayer), which picks out some important aspects of control. E. H. Roberts and R. D. Smith have written a clear account in support of their hypothesis that the pentose phosphate pathway plays a central role in

the breaking of dormancy. Although their proposals are of considerable interest their data fall short of being conclusive for any species and it may be premature to consider the applicability of the hypothesis to a wide range of species. Unfortunately the book ends on a low note in a poorly written chapter on "Hormonal Regulation of Nucleic Acids and Proteins in Germination", by K-L. Tao and A. A. Khan, which seriously overlaps some earlier chapters and which provides a badly expressed jumble of information. Any glutton for punishment who reaches the top of p423 will find the following sentence: "Isocitratase is an important enzyme in Krebs cycle."

The quality of the book is slightly marred by the occurrence of rather more typographical errors than one would expect. It is a pity that there is no author index; had space been saved by the provision of one consolidated list of references, an author index could have been produced without any increase in pagination. However, the faults seem fairly negligible when balanced against the general excellence of the work.

J. W. Bradbeer

J. W. Bradbeer is Professor of Botany at King's College, University of London, UK.

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